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Rice (Oryza sativa. L) genetic diversity for early vigor and drought tolerance at the vegetative stage: Identification of morphogenetic, metabolic and hydraulic traits towards genetic studies

Diversité génétique de la vigueur initiale et de la tolérance au stress hydrique chez le riz (*Oryza Sativa*.L) : Identification de caractères morphogénétiques, métaboliques et hydriques pour les études génétiques

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Introduction

CONTEXT

Hosting projects

This PhD work was part of two larger commissioned research projects: Orytage (CIRAD internally funded project; Centre de Coopération Internationale de Recherche en Agronomie pour le Développement) and Genphen (GCP project; funded by Generation Challenge Program of the CGIAR, project coordinated by IRRI, International Rice Research Institute). Both projects aimed at phenotyping rice (*Oryza sativa* L.) diversity panels for plant performance (yield in Genphen, vegetative growth in both projects) under non limiting conditions (Orytage) or abiotic constraints (drought in both projects) to conduct genetic association studies. The challenge of these projects is to take advantage of a chip of about 1 million Single Nucleotide Polymorphism (SNP) markers, currently in the testing phase, covering the entire rice genome (available in 2012). Association studies using whole-genome, saturating genotyping methods are expected to be extremely powerful to indentify genomic regions and eventually the allelic diversity of genes controlling traits of interest in rice panels. For this purpose, complex traits characterizing plant performance have to be dissected into elemental, process based traits controlled by a smaller number of genes and possibly, prone to less variation caused by experimental noise, environment and Genotype by Environment (GxE) interactions.

One common focus of the funded projects was the phenotyping of early morphogenesis, early vigor and their expression under drought within the japonica subspecies. Also, new phenotyping tools and approaches were needed in order to quantify process based traits involved.

Rice aerial vegetative morphogenesis

Rice is an annual C3 crop of the *Poaceae* family. Its ontogenesis is defined by the succession of a vegetative stage (including germination, seedling emergence and growth, until panicle initiation), the reproductive stage (panicle differentiation, stem elongation, heading and flowering), and finally grain filling and maturation. Rice vegetative morphogenesis consists of the development and growth of successive phytomers (made of a leaf, node, internode, axillary bud and potentially, roots; (Nemoto *et al.* 1994). Leaves present an alternate phyllotaxy. Primary tillers appear from axillary buds at the basis of each leaf on the main stem (or main tiller). Tillers can produce tillers of second order and so

on **(Figure 1)**. A leaf is considered as fully developed (ie. ligulated) once its ligule (thin membranous structure at the intersection of leaf blade and sheath) and the tip of the next (expanding) leaf has appeared. On the main stem, the first leaf that appears lacks a leaf blade and is called prophyll. Thus the second leaf, i.e. the first with differentiated blade and sheath, is counted as the first one **(Figure1).**

On cereals, the time separating the visible (macroscopic) appearance (i.e. tip appearance from the enclosing sheath of the previous leaf) of two consecutive leaves on the main stem is called **phyllochron**. The **plastochron** is the time separating the initiation of two consecutive leaf primordia at meristem (microscopic) level (Klepper *et al.* 1982). In rice phyllochron and plastochron are closely synchronized, which enables using phyllochron as a proxy for plastochron, at least in the absence of stresses (Nemoto *et al.* 1994; Itoh *et al.* 2001). The phyllochron has also been extensively used as a unit of physiological age to characterize and model shoot structural development (Haun 1973).

During vegetative growth, plant leaf area increase is composed of individual leaf size and leaf and tiller emergence rate. It determines the capacity of the plant to intercept radiation and produce biomass, which impacts on yield later on during the reproductive and grain filling phases. Tillering rate set the number of fertile tillers and thus of panicles at maturity (Yoshida 1981).

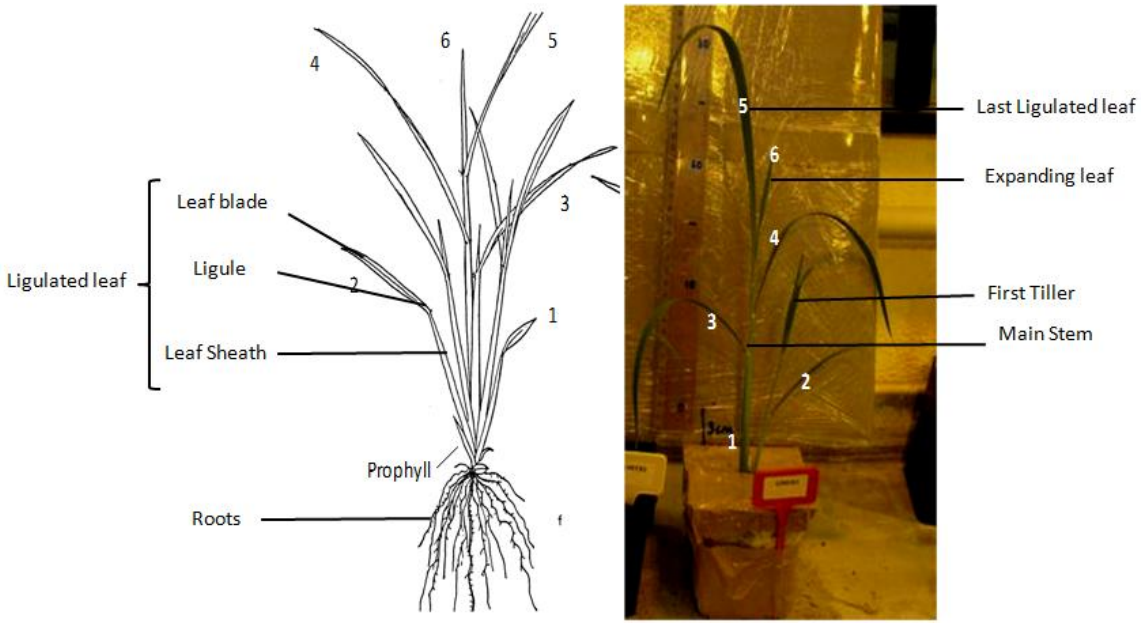

Figure 1 Rice plant at the vegetative stage; the rank of ligulated leaves (1 to 5) and expanding leaf (6) on the mains stem is indicated. First primary tiller, growing from the axillary bud of phytomer 1 on the main stem is also indicated. Schematic diagram on the left from (Yoshida 1981); picture on the right from greenhouse experiment described in chapters I and II.

Rice crop performance under drought: implications for breeding and genetic diversity

Because of its semi-aquatic environment of origin, rice is commonly considered to be poorly adapted to water limited conditions (Lafitte *et al.* 2006). However, *Oryza sativa* L., the main cultivated rice specie, has been progressively selected in the domestication process for different agro-ecological systems with contrasting water managements. Upland environments are free-draining, aerobic and usually rainfed. Lowland environments, which are at least temporarily flooded (anaerobic), can be rainfed, naturally flooded in the case of flood plains, or irrigated. This resulted in a large genetic diversity in terms of morphology and drought adaptation (Courtois *et al.* 2000; Robin *et al.* 2003; Liu *et al.* 2004). Drought can occur in both upland and non-irrigated lowland systems. Rice is thus well adapted to either anaerobic and aerobic situations, or both in alternation (Wade *et al.* 1998).

Improving crop performance under drought, particularly for food crops, is a global issue because of the increasing frequency and intensity of drought events associated to climate change (Mpelasoka *et al.* 2008; Wassmann *et al.* 2009), particularly in regions with uncertain food security. Furthermore the competition for water resource between agricultural and other uses implies that water consumption needs to be optimized (Condon *et al.* 2004). Rice is a staple crop, with a world production in 2010 of 672 Mt on a cultivated area of 153 MHa (FAOSTAT 2011); it consumes 30% of the world's developed fresh water resource (Bouman *et al.* 2007). Higher rice productivities have been attained in irrigated environments by improving yield potential and reducing crop duration, whereas less success have been made in drought prone environments such as rainfed cropping systems (Kamoshita *et al.* 2008; Serraj *et al.* 2008a). Large rice production areas in Asia and the majority of rice production areas in Africa and Latin America are based on rainfed water management **(Figure2)**. Therefore, there is an increasing need of better adapted cultivars combining improved yield potential and lower drought sensitivity (Lafitte *et al.* 2006; Kumar *et al.* 2008). Cultural practices such as dry direct seeding are increasingly used to substitute for transplanting in lowland systems in order to increase rice water productivity and reduce unproductive components of the water balance such as surface evaporation and deep percolation. However, this practice favors weed development in the field and the occurrence of abiotic stress, in particular drought periods (Farooq *et al.* 2011). Combined efforts must be made to breed for rice varieties with better competition against weeds and a rapid access to soil water resources during the vegetative phase to secure grain yield (Kumar *et al.* 2009; Okami *et al.* 2011).

Rice is predominantly autogamous and hence natural gene flow is restricted. As a result of geographical or ecological division, genetically distinct groups of rice exist (Garris *et al.* 2005). An isoenzyme classification (Glaszmann *et al.* 1984) divided cultivated rice into 5 genetic groups: indica, tropical japonica, temperate japonica, aus and aromatic. Fukai *et al.* (1995) reported that in general

there is a poor allelic diversity on loci present in rice improved varieties. There is thus an increasing interest in characterizing the genetic diversity within genetic groups including landraces and traditional cultivars adapted to the different agro ecological conditions. The tropical japonica type, more adapted to aerobic conditions, might provide a good potential for increasing rice drought resistance (Lilley *et al.* 1996; Courtois *et al.* 1999). Allelic diversity translates into polymorphisms at the molecular level. **Diversity panels capturing a wide polymorphisms for relevant genes are suggested to serve the initial exploration of useful diversity, as a first step to gene mining through genetic marker assisted trait pyramiding and selection (McNally *et al.* 2009; Jahn *et al.* 2011; Xu *et al.* 2011).**

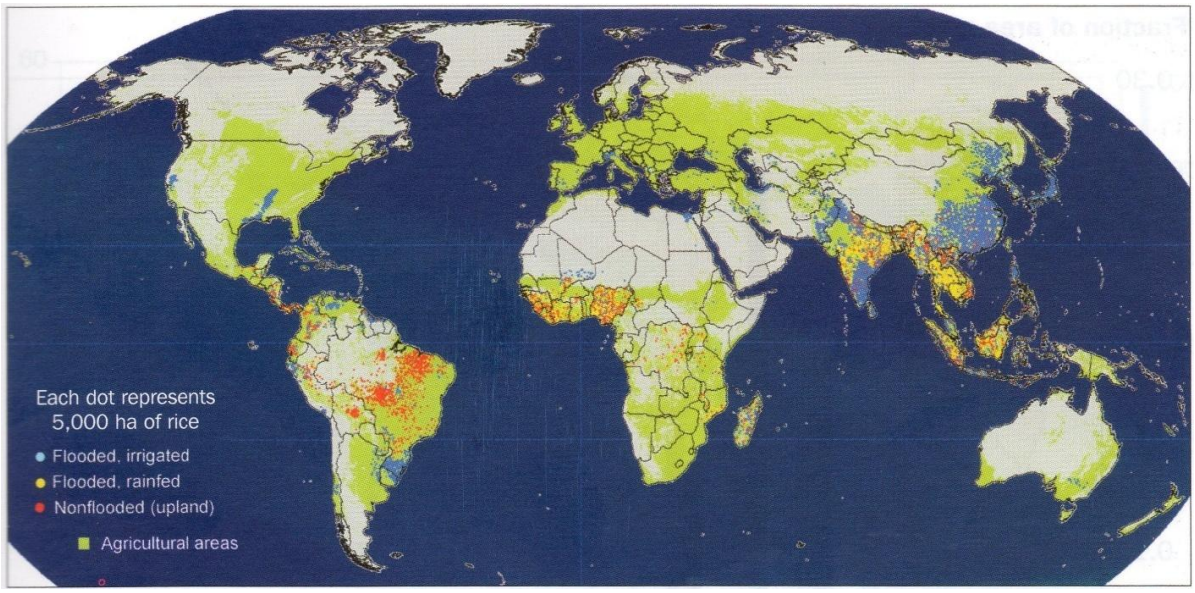

Figure 2 *Rice cropping area for major ecosystems (Hijmans et al. 2008)*

Role for early vigor in rice crop performance

Early vigor is the ability of annual plants to rapidly accumulate biomass and leaf area until canopy closure. It is an emergent property resulting from many processes including resource acquisition and conversion, organ and morphogenetic dynamics, plant and canopy architecture. It favors a rapid colonization of space and resources (Asch *et al.* 1999; Dingkuhn *et al.* 1999). Early vigor can thus contribute to improving yield stability, for example in drought prone environments. By contributing to early canopy closure, it also reduces unproductive, non-transpirational water use and thus increases overall crop water use efficiency (WUE) (Condon *et al.* 2004). WUE is indirectly enhanced by the improved weed competitiveness of the crop conveyed by early vigor (Dingkuhn *et al.* 1999; (Zhao *et al.* 2006b; Zhao *et al.* 2006c). Lastly, early vigor is an essential trait for enabling high yields in short-duration varieties, and short duration directly translates into lower overall water consumption.

Early vigor was also related to low yield, due to small grains and lodging propensity due to plant height (Zhao *et al.* 2006c). Besides as a result of the selection pressure for semi-dwarf, high yielding genotypes, early vigor has been reduced (Cairns *et al.* 2009). However (Zhao *et al.* 2006b; Namuco *et al.* 2009) reported that early vigor and high yield can be combined, suggesting these complex traits do not present a systematic, negative (physiological or genetic) linkage.

Limited progress has been made in increasing early vigor in rice (Cairns *et al.* 2009) although it has been a breeding objective for upland rice (Dingkuhn *et al.* 1999) or wheat (Botwright *et al.* 2002; Richards *et al.* 2010). Early vigor is characterized by complex traits such as plant biomass accumulation or leaf area expansion during seedling exponential growth. These integrative traits are strongly prone to GxE as they result from the interaction of multiple mechanisms controlled by a high number of genes (Chapman *et al.* 2003). This makes their genetic study difficult. Moreover some component traits themselves can be prone to strong GxE such as Specific Leaf Area (SLA) or leaf number (Rebetzke *et al.* 2004; Rebetzke *et al.* 2008). **Consequently, in order to improve the genetic study and improvement of early vigor through breeding, efforts are still to be made to define elemental traits, less prone to GXE interactions and identify the nature of the linkages among them.**

Regarding drought prone environments, early vigor may contribute to drought avoidance through more vigorous root systems, but it is not an avoidance mechanism *per se*. It thus has to be combined with drought tolerance mechanisms (Reynolds *et al.* 2007). Characterizing the drought type the rice crop is exposed to (i.e. the TPE: Target Population of Environments; Heinemann et al. 2008) can help defining appropriate breeding criteria for drought adaptation, notably traits that contributing to yield stability under drought (Tardieu *et al.* 2010; Sinclair 2011). Indeed, while breeding for rice early vigor and deeper root systems for improved access to water was successful in rainfed lowland cropping systems with deep soils (Wade *et al.* 1998), it was much less effective in soils with a high resistance to root penetration or in shallow soils (for example acid soils) inhibiting the expansion of the root system (Heinemann *et al.* 2008; Cairns *et al.* 2011). **Breeding rice for a combination of early vigor and drought tolerance is thus an important objective.**

Morpho-physiological mechanisms underlying plant early vigor

Carbon related traits: assimilation rate and partitioning

Plant biomass accumulation can *a priori* be directly attributed to carbon (C) assimilation; this was reported for rice seedlings (Baker *et al.* 1990). During seedling growth, all assimilates not invested in the root are allocated to leaf structure. Therefore traits related to light interception (plant leaf area and geometry) and conversion into structural biomass should explain most of the genotypic differences in terms of biomass accumulation and thus early vigor, as long as soil-borne resources (water and nutrients) are not limiting. All traits contributing to resource acquisition are of interest to improve early vigor: water and nutrient uptake (i.e. root system extent and depth, uptake capacity) and net assimilation (physiological nitrogen use efficiency, leaf chlorophyll content, Rubisco activity, SLA, stomatal conductance, leaf senescence, respiration rate).

During vegetative growth, C assimilates are translocated from source (autotrophic leaves) to sink organs: meristems and expanding organs (the latter growing in the previous leaf's sheath and gradually attaining autotrophy upon appearance) or (ii) storage organs (i.e. leaf sheaths for rice; since internodes are not present during vegetative development). The way the rice plant allocates available C assimilates to sink or storage organs can differ among genotypes (Dingkuhn *et al.* 2007) and environment, in order to maintain a functional equilibrium (Brouwer 1983; Reich 2001; Shane *et al.* 2003). Consequently**, not only source but also sink strengths are drivers of genotypic differences in biomass accumulation and early vigor**(Dingkuhn *et al.* 2007)**.**

Carbon sink related traits

Meristem (ie. Sink) activity determine primordium initiation rate, cell division and expansion. In rice seedlings sink activity at shoot level can be quantified by the phyllochron or its inverse, the Developmental Rate (DR: 1/phyllochron), leaf size and expansion rate, and tillering. All can be considered as sink component traits of early vigor and contribute to biomass accumulation. However they compete for the same resource pool, in particular C assimilates (Dingkuhn *et al.* 2005). Negative linkages between sink component traits, such as organ size and number, were reported in previous studies on several species, for example on wheat (ter Steege *et al.* 2005) and *Arabidopsis* (Tisne *et al.* 2010). Tisne *et al.* (2010) suggested that this linkage was partially of genetic nature. Such negative linkages make more complex the combination of sink related traits to improve early vigor. However, for rice it was suggested by Miyamoto *et al.* (2004) that the positive, physiological linkage between phyllochron and tillering does not have genetic bases. **These studies emphasize the need of**

considering traits in an integrative way rather than individually to understand the genetic bases of early vigor (Granier *et al.* 2009).

Dingkuhn *et al.* (2007) postulated that rice vegetative growth is mainly driven by sink dynamics under non limiting environmental conditions and presented accordingly a plant growth model Ecomeristem validated on rice (Luquet *et al.* 2006). Ecomeristem was initially developed to simulate rice vegetative growth and its plasticity in response to environmental conditions (water, light, temperature). The main concepts of Ecomeristem rely on the existence of a two way interactions between growth and development processes, and of transitory C reserves as a buffer (Dingkuhn *et al.* 2007). In Ecomeristem sink capacity is determined by genotypic parameters defining potential meristem activity. Other genotypic parameters define the reaction norms of meristem activity for different environmental conditions, and more particularly plant nutritional status. These reaction norms feedback and regulate meristem activity, thus on morphogenesis, generating phenotypic plasticity (Granier *et al.* 1999; Luquet *et al.* 2008a; Lafarge *et al.* 2010)**.These modeling concepts illustrate the way traits constituting plant early vigor and its plasticity can be considered in an integrative way and the role modeling can play for its study (Dingkuhn *et al.* 2005).**

Early vigor response to drought: regulation of source-sink relations for C and water

Drought response mechanisms

Multiple mechanisms are involved in plant, particularly rice, response to drought and can be divided in drought escape or drought resistance traits. From an agronomic point of view, an effective drought resistance trait should allow to reduce plant physiological stress and maintain growth under drought. Drought resistance mechanisms enabling growth maintenance by a reduction of plant dehydration can be defined as **avoidance** mechanisms following Levitt (1985). Drought avoidance traits identified in rice are involved in improved water uptake (deep rooting, high root density, high hydraulic conductance; (Nguyen *et al.* 1997; Courtois *et al.* 1999; Price *et al.* 2002) or reduced water loss (small leaf area, high stomatal resistance; (Cabuslay *et al.* 2002; Serraj *et al.* 2008b). In rice the genetic bases of root related traits improving drought avoidance were reported (Price *et al.* 1999; Courtois *et al.* 2000) and these traits were suggested as effective mechanisms to maintain yield in drought prone environments (Serraj *et al.* 2009).

Drought resistance traits allowing the plant to keep on growing and use available water under high dehydration are named drought **tolerance** mechanisms. In rice, the genetic variation for shoot traits such as leaf rolling (Dingkuhn *et al.* 1999) or osmotic adjustment (Fukai *et al.* 1995; Lilley *et al.* 1996),

that enable leaf turgor and stomatal conductance maintenance under drought, have been reported. Traits favoring the maintenance of cellular functioning in a situation of dehydration (membrane integrity, maintenance of the enzyme activity involved in Carbon fixation, low injury caused by reactive oxygen species) were recently reviewed by Blum (2009) and Farooq *et al.* (2009). Drought activates signaling cascades that results in changes in plant metabolism including: the activation and synthesis of antioxidants, the synthesis and accumulation of osmo-protectors and stomatal closure. All these processes control growth maintenance under drought.

Although the physiological role of drought tolerance processes was already reported (Lilley *et al.* 1996), their genetic study is still scarse in rice. Price *et al.* (2002) insisted on the need to deal with QTL (Quantitative Trait Loci) for rice shoot tolerance traits to improve drought resistance in complement of QTLs related to root morphological traits and drought avoidance, based on the genetic study of a mapping population from the cross between Bala and Azucena genotypes. **Tolerance and avoidance mechanisms** were reported to have different genetic control in rice (Yue *et al.* 2006), suggesting their **association is possible in order to optimize the improvement of plant growth under drought (Price *et al.* 2002).**

Water and C balance relations along rice shoot growth: implications for growth maintenance under drought

In order to isolate drought tolerance traits and explore their impact on growth maintenance under drought, it must be first considered that plant growth under drought results from constitutive (capacity or potential expressed under well watered conditions) and adaptive (regulation expressed under water deficit) processes (Liu *et al.* 2004; Kamoshita *et al.* 2008). Both constitutive and adaptive traits contribute to an effective use of water (Blum 2009) and C assimilates for growth.

Luquet *et al.* (2008) and Parent *et al.* (2010) reported that the reduction of leaf growth under drought was associated with a reduction of transpiration rate (thought stomatal closure) in response to decreasing soil water availability, and Abscisic Acid (ABA) production (Liu *et al.* 2005). Stomatal closure under drought reduces water loss and, in the same time, CO_2 absorption and thus C assimilation. Indeed, its role in equilibrating growth maintenance and water conservation is a major issue for breeding (Condon *et al.* 2004) since it controls both; the way plant conserves water and maintains C assimilation. Water loss through leaf cuticle and the boundary layer can also impact on plant dehydration, thus on C assimilation and growth under drought (Farooq *et al.* 2009).

Photosynthesis is one of the primary physiological processes strongly affected by drought (Muller *et al.* 2011) and the genetic control of photosynthesis under drought in rice has been recently reported by Gu *et al.(*2012). Conversely studies' focusing on plant C assimilates partitioning from source to sink organs; its regulation and relation with drought tolerance are scarce (Farooq *et al.* 2009). Growth maintenance under drought depends on the capacity of sink organs to keep on using incoming C assimilates from source organs (Roitsch *et al.* 2000; Stitt *et al.* 2007), which can severely affect growth despite no C starvation is observed (Muller *et al.* 2011). Luquet *et al.* (2008) reported on one indica rice genotype that C assimilates were not the limiting factor for the seedling until a severe level of stress. The same authors demonstrated different concentrations in non structural carbohydrates (starch, hexose, starch) under drought depending on the leaf in which they were measured (source: expanded leaf or sink: expanding leaf). In particular, starch concentration decreased while hexose increased and sucrose was maintained in source leaves. The opposite was observed in sink leaves and roots, suggesting sugar types and their dynamics in sink organs are relevant metabolic markers for rice seedling growth and sink activity response to drought. **Therefore non structural sugars should be relevant metabolic markers for phenotyping the source-sink relation underlying rice early vigor under drought, in complement to traits related to water use**.

Opportunities and limits to phenotype process based traits of early vigor and drought response

Recent advances in genetics and genomics (high-throughput sequencing and genotyping technologies (Miura *et al.* 2011), particularly for rice, a model crop for the genetics of cereals, C3 and monocot plants, provide the opportunity to accelerate the breeding process. Indeed molecular markers related to relevant traits (eg. drought tolerance) can be used to improve the performance of existing high yielding elite cultivars (Roy *et al.* 2011).

High throughput phenotyping tools for hundreds or even thousands of genotypes allow to compare genotypic responses in similar environmental conditions (Sinclair 2011). Indeed, building large experimental areas with homogenous and controlled environmental conditions and the automation of non invasive measurements, nutrient and water inputs provided a solution and are performed on recent phenotyping platforms (Granier *et al.* 2006). Ideally these methodologies would have to be as cheap as possible and easy to transfer as a breeding technique.

However, to identify traits of interest for molecular markers **the development of phenotyping tools did not progress as fast as molecular genetics**, despite of real advances (Furbank *et al.* 2011). Traits genetically simpler than complex traits defining plant performance (grain yield, biomass) and closer to gene action (less noised to GxE), i.e. **process based traits**, **are needed for their high throughput**

estimation. The identification of relevant process based traits for genetic studies was thus a major challenge during the last decades for ecophysiologists (Dingkuhn *et al.* 2006; Tardieu *et al.* 2010).

Recently some approaches proved their relevance for phenotyping process based traits on a large number of genotypes. This is the case of high throughput imaging methods that enable a rapid characterization of plant growth dynamics or water status (Berger *et al.* 2010). The application of modeling also showed its relevance to phenotype process based traits using the parameters of equations formalizing plant response to environmental variables to differentiate genotypes; such parameters are thus genotypic and less prone to GxE than corresponding measured variables (Dingkuhn *et al.* 2006; Tardieu *et al.* 2010). This was pioneered by Reymond *et al.* (2004) using a simple model (with 3 equations) predicting maize leaf expansion rate (LER) response to environmental variables related to drought conditions (leaf temperature, vapor pressure deficit and soil water potential). Indeed consistent associations of Quantitative Trait Loci (QTL) and model parameters were reported for hundreds of genotypes in maize populations with different genetic backgrounds and across environments (Welcker *et al.* 2011).

The interest of metabolomics for plant phenotyping has been less explored (Fernie *et al.* 2009), although they might reveal genotypic variation for growth and adaptation strategies (Stitt *et al.* 2010) or for physiological traits (Ishimaru *et al.* 2007). The role of non structural carbohydrates (NSC) as markers of genotypic growth pattern was demonstrated: on Arabidopsis, Sulpice *et al.* (2009) reported a negative correlation between seedling growth and starch accumulation and on Medicago trucatula Vandecasteele *et al.* (2011) reported a negative correlation between seedling vigor and sucrose:rafinose ratio. Meanwhile in a preliminary study on 200 rice sativa genotypes grown under well watered conditions Luquet *et al.* (2008b) reported that early vigor was associated to low NSC concentration in the plant. Metabolic component traits demonstrated also their interest to discriminate genotypes for drought response mechanisms (Shao *et al.* 2009; Verslues *et al.* 2011), rice vegetative growth (Cabuslay *et al.* 2002). **This suggests the relevance of exploring the genetic diversity for NSC during rice vegetative growth.**

OBJECTIVES, OVERVIEW OF THE CHAPTERS AND THE METHODOLOGY

Within Genphen and Orytage projects, extensive phenotypic data sets were generated with a threefold purpose (i) identify traits contributing to rice growth and adaptation strategies (to drought in particular) and their diversity, (ii) characterize physiological and/or genetic linkages among traits, and their GxE; and (iii) detect QTLs and related favorable alleles for studied traits using a chip of about 1 million SNP markers for association genetics studies. Genetic studies are planned for June 2012 when the chip of SNPs markers will be available.

This PhD work focuses thus on points (i) and (ii). **It aims at exploring traits underlying the genetic and phenotypic diversity of rice early vigor and its maintenance under drought** in a panel of 200 genotypes representing the diversity in the tropical Japonica subspecies**.** Studied traits should enable the **genotypic discrimination for process based traits or reaction norms**, i.e. the response of a biological process to environmental conditions or plant nutritional status, in particular related to C and water resources; this should be performed with a **phenotyping approach** i.e. on a large number of genotypes constituting studied diversity panel and in a minimum of time.

The first hypothesis to validate is that genotypic variation in early vigor can be explained by variation of sink related traits, i.e. that high constitutive vegetative growth is more sink than source driven. The first chapter entitled "**Developmental dynamics and early growth vigor in rice. I. Relationships between developmental rate (1/Phyllochron) and growth"** addresses this hypothesis under well watered conditions. It explores the role of plant organogenetic developmental rate (DR: 1/ phyllochron) as a driver of early vigor, conjointly with leaf size and tillering, using data collected in Montpellier in a greenhouse in 2009 (during this PhD) on a japonica panel of 200 genotypes and available data previously collected in the field in IRRI in 2006 on another panel of 200 sativa genotypes. A complementary study was carried out based on a modeling approach, using Ecomeristem model to further explore the C source-sink relationships underlying the role of DR on rice early vigor, as experimentally observed on the japonica panel in Montpellier. The use of the model was not included *per se* in the PhD work but approaches were strongly interconnected to address above-mentioned hypothesis which resulted in many interactions with modelers (**AppendixI**).

The first hypothesis is addressed under drought conditions in chapters II and III that also aim at exploring the relationships between early vigor, metabolic and drought tolerance traits.

In the second chapter:"**Phenomics of rice early vigor and drought response: Are sugar related and morphogenetic traits relevant?":** the way early vigor is related to genotypic differences in morphogenetic and metabolic (sugar) traits is addressed in a subset of 43 japonica genotypes, as well as the way morphogenetic traits described in chapter 1 are related to metabolic traits (soluble sugars, starch) in source and sink leaves, under well watered and drought conditions. This study enables addressing also the hypothesis that rice seedling under drought are not C source limited but rather sink limited (Luquet *et al.* 2008; Muller *et al.* 2011).

The third chapter "**Does early vigor occur in combination with drought tolerance and efficient water use in rice?" explores morphogenetic traits are related to hydraulic limitations (transpiration rates).** In this part another data set, acquired in a greenhouse experiment at IRRI in 2010 on the

same panel of 200 japonica genotypes, is used to confirm the genetic diversity of morphogenetic, constitutive and drought response traits reported in chapter 1 and 2. **More particularly, this chapter aims at evaluating the genetic diversity of water related traits under drought (plant leaf transpiration rate and efficiency) and their linkage with morphogenetic traits.**

Finally, the fourth chapter is a general discussion of this work. Considering the results of Montpellier and IRRI experiments in chapters I, II, III, **it addresses the opportunities and limits of the present work regarding the phenotyping of early vigor diversity and its drought response, as well as the consequences for genetic studies and finally molecular breeding.** Future physiological and genetic studies are suggested to better understand trait interactions that limit the improvement of rice early vigor and the consequence for grain yield, under well watered and drought conditions .

Methodological overview: details on the dry-down system

To our knowledge few studies have focused on drought tolerance traits avoiding the expression of rooting (i.e. avoidance) differences during the vegetative stage (Kato *et al.* 2008; Luquet *et al.* 2008; Parent *et al.* 2010). For controlled environments, Luquet *et al.* (2008) presented a dry down methodology that enables simultaneous monitoring of drought establishment, dynamically measuring plant growth and avoiding the expression of rooting differences by a reduced soil volume in pots. This gravimetric dry down system was adapted (and semi-automated) for a large diversity panel (200 genotypes). The use of this system to monitor and compare drought impact on several genotypes used the Fraction of Transpirable Soil water (FTSW) as an indicator for drought intensity, based on the hypothesis that plants respond similarly to the progressive soil drying across a wide range of conditions (climate, soil type, pot size) when water stress is expressed on the basis of FTSW (Sinclair *et al.* 1986; Sinclair 2005). To adapt the dry down system detailed by Luquet *et al* (2008) to a large panel we calculate FTSW', by contrast to FTSW, with FTSW' considering the same wilting point value for all genotypes.

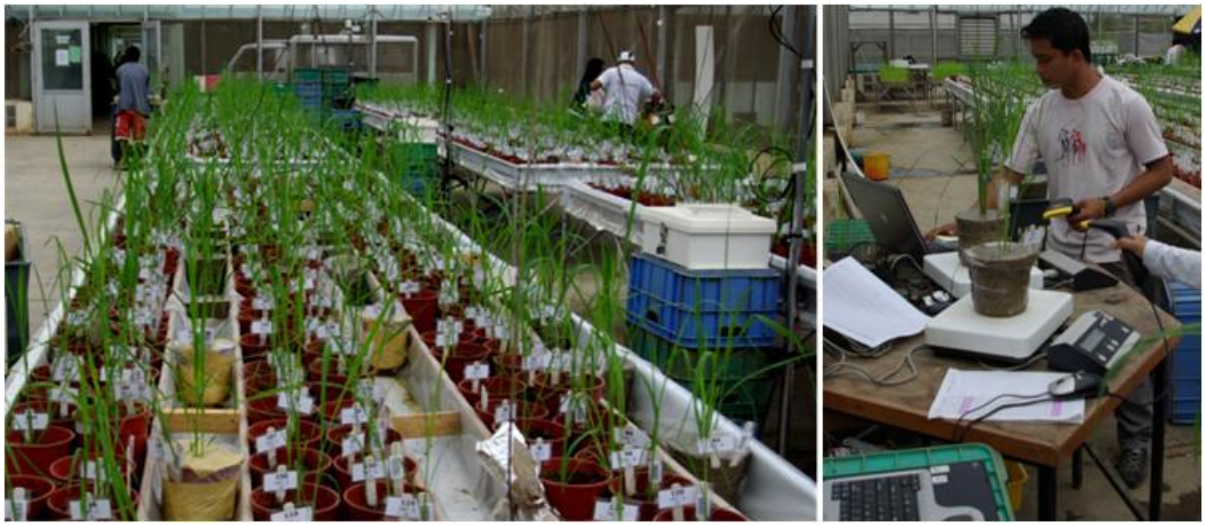

Figure 3. *Dry-down experimental design in a greenhouse at IRRI (Los Banos, Philippines) from September to November 2010: (left) irrigated and packed (stressed) plants of a given genotype are placed on the same table but separated by a small wall; (right) daily weighing of stressed plant pots and data registration using a bar code scanner.*

Water depletion and thus stress intensity was measured gravimetrically from stress onset (FTSW' 1) at 6th leaf stage on the main stem **(Figure 1)**. This stage was chosen since it is representative of the exponential growth phase of seedlings before panicle initiation.At this stage it was checked that seedling roots explore the whole soil volume contained in 1 liter pots. Therefore this system avoids the expression of genotypic differences in plant water availability and uptake (ie. drought avoidance mechanisms). The day before stress onset, the soil was fully re-watered from the top and then drained overnight. The following morning pots were entirely covered with a plastic film to prevent soil evaporation and enable water loss only by plant transpiration. Pots were then weighed every morning before 10AM. Pots of the well watered treatment were maintained in a flooded table but were not submerged by the top. Two dry down experiments were performed during this PhD **(Table 1)**. Before each experiment, soil type and pot size was tested to check (i) if roots explored all the pot volume for a few contrasted genotypes (in terms of vigor) at 6th leaf stage; (ii) if the dry-down was slow enough in the chosen soil volume and thus if it was possible to observe gradual plant response to stress establishment until FTSW' 0.2; and (iii) the values of soil water content (humidity) at field capacity and wilting point, needed for FTSW' computation.

In Montpellier (Exp1 in 2009), only two temporal replications were considered (for each genotype and water treatment). The reason is that we encountered experimental problems with the environment monitoring system in the greenhouse during the third replication. Therefore the same experiment was performed at IRRI in 2010 (initially a reproductive drought phenotyping was

planned). The final harvest at FTSW' 0.2 (targeted level of stress) was used to characterize shoot dry weight SDW and analyze soluble sugar and starch content in source (last ligulated) and sink (hidden, expanding) leaves. Plants were sampled before 10AM. A FTSW' of 0.2 corresponds to a predawn leaf water potential of -0,8MPa (measured on exised ligulated leaves for one rice genotype with a pressure chamber), thus considered as a severe stress, for the soil used in Montpellier. The method used for sugar content analysis was based on HPLC chromatography for soluble sugars and enzymatic analysis for starch content detailed in (Luquet *et al.* 2006). This method was not adapted to analyze a large number of samples. Therefore only 43 genotypes were considered in a first step. These metabolic analysis also shed light on assimilate abundance (source status) of the plant. Indeed, we did not have the resources to measure assimilation rates on the entire panel. This was only performed on 8 genotypes for the two replications in Montpellier to check for genotypic differences under well watered and drought conditions. The results were not used in this thesis because no such differences were observed at statistically significant levels.

At IRRI (Exp2), three simultaneous replications were monitored for all genotypes and treatments; the experimental design was a bit improved compared to Montpellier (Exp1): stressed and well watered plants were placed side to side in the same table **(Figure 3)** and additional shoot biomass sampling was performed before stress application as well as a measurement of plant leaf area at final sampling. During the dry-down, to optimize daily gravimetric data registration for each stressed plant, pots were tagged with a barcode. This allows a daily pot weight recording, instantaneous computing of FTSW' and thus a precise final plant harvest at an FTSW' value of 0.2. Daily plant leaf transpiration was calculated as the pot weight difference between two successive days and was normalized by plant leaf area (cm^2), measured at final sampling and extrapolated to the days before (as explained in Chapter III) and potential evapo-transpiration (PET in mm)computed afterwards (see details in Chapter III).

Table 1 Environmental characteristics during greenhouse experiments: experiment 1 in Montpellier and 2 at IRRI (Los Banos, Philippines).

Experiment	Characteristics	Growing period	Temperature (°C) (Min- Max)	Humidity (%) (Min- Max)	RG (MJ/m2) (mean)	Soil Properties (1 litter pot)
Experiment 1						
Montpellier France	2 repetitions	09/02/2009 to	22-33	27- 62	4,59	wp:0,11; Fc: 0,59
3,9° 43,6' alt: 20	2treatments	12/05/2009				Silty Clay Loam Soil (451g)
Experiment 2						
Los Banos Philipinnes	3 repetitons	16/09/1010 to	25 - 36	65 - 79	6,97	wp: 0,15; Fc: 0,62
14° 10' alt: 66	2 treatments	05/11/2010				Upland Soil (930g)

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Headlines in the introduction part:

- Early vigour is essential for rapid crop establishment and resource acquisition (light, water and nutrients), and by consequence for competition with weeds. It contributes to improve yield potential and stability in drought prone environments.

- Rice is known to be a poor competitor, particularly in poorly controlled systems prone to weeds and abiotic stresses.

- The tropical japonica genetic group has a largely unexploited genetic diversity for traits related both to early vigour and drought tolerance.

- Early vigor depends on the rate at which seedling uses resources to grow. Drought affects both Carbon (C) resource acquisition and sink dynamics (leaf expansion, size and tillering). Early vigor plasticity depends on plant's inherent capacity to regulate these source-sink dynamics.

- Discovery of physiological component traits for a complex trait, such as early vigor and its response to drought, now integrates:
  - Exploration of available trait diversity through phenotyping
  - Information on traits genetics through association analysis
  - Association of component traits with complex traits on a phenotypic basis (existent combination of traits, traits interactions) and on a genetic basis (co-localization).

- Integrative studies using metabolic and morphogenetic traits can potentially contribute to understand shoot morphogenesis plasticity under drought.

- The bottleneck in this integrated approach is the availability of reliable, rapid and for some cases cost effective phenotyping methodologies for component traits under drought.

- This Ph'D work aims at characterizing, in a tropical Japonica diversity panel, the diversity of morphogenetic, metabolic and hydraulic traits constituting early vigor and its plasticity under drought, under a short and severe water deficit.

Chapter I: Developmental dynamics and early growth vigor in rice. Relationship between Developmental rate (1/phyllochron) and growth

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Running Title

Developmental dynamics and early growth vigor in rice

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ABSTRACT

Achieving early vigor, i.e. rapid dry matter accumulation is a priority in rice crop improvement, but this trait is complex and not well understood genetically and physiologically. This study tested the hypothesis that developmental rate (DR) contributes to early vigor.

Two diversity panels were phenotyped during vegetative stage for traits constituting early vigor (shoot dry weight, SDW; relative growth rate, RGR), or contributing to early vigor (tillering, leaf size, DR). The first panel comprised 169 genotypes from all major genetic groups of O. *sativa* and was phenotyped under irrigated upland conditions in the field (Philippines, Exp1). The second panel with 190 genotypes representing the diversity of the tropical japonica group was phenotyped in pots in a greenhouse (Montpellier, France, Exp2).

Results from field and pot experiment pointed out that DR, tillering and leaf size were positively correlated with RGR and SDW, although the contribution of leaf size was small. DR was positively correlated with tillering but both were negatively correlated with leaf size. DR *vs*. RGR correlation was conserved in subsets of genotypes with similar leaf size and tillering, suggesting an effect of DR on RGR independent of the other traits.

DR is a promising, still underexploited trait contributing to rice early vigor, requiring further genetic and physiological characterization.

INTRODUCTION

Early vigor contributes to rapid crop establishment and resource acquisition (light, water and nutrient capture). It is particularly useful i) in low-input systems such as conservation tillage or organic agriculture where no herbicides are used, to provide the plant with a good competitiveness against weeds; ii) as a compensation for short crop duration, a trait generally sought after by breeders; and iii) as a compensation for low seed rates if seed is expensive and needs to be sown at low density (e.g., commercial hybrids). Early vigor is critical in several rice ecosystems, namely irrigated systems using direct seeding (Dingkuhn *et al.* 1990; Dingkuhn *et al.* 1991), rain-fed lowland systems having poor water control, implying for seedlings to rapidly colonize soil and aerial space to access resources (Jongdee *et al.* 2006) and rain-fed upland systems that are affected by an aggressive and drought tolerant weed flora (Dingkuhn *et al.* 1999; Suzuki *et al.* 2002; Caton *et al.* 2003).

Early vigor can be defined as the plant's ability to develop leaf area rapidly during early development. It is a major selection criterion in rain-fed rice breeding, commonly assessed visually by breeders in the field without considering its morphological or phenological causes. For wheat, (Rebetzke *et al.* 2007) suggested that the length of the coleoptile and the width of the second leaf were heritable traits related to early vigor. According to Lopez-Castaneda et al. (1996), embryo size is the seed property that is most predictive of early vigor in several cereal species. (Dingkuhn *et al.* 1999) suggested that high specific leaf area (SLA, $cm^2\ g^{-1}$) conveys superior weed competition in upland rice. The involvement of SLA and several other morphological traits of the seed and seedling in early growth vigor was confirmed recently for a broader range of rice cultivars (Namuco *et al.* 2009).

In terms of functional ecology, early vigor is part of the adaptation strategy of fast-growing, annual, frequently ruderal plants. It is characterized by high relative growth rate (RGR, $g\ g^{-1}\ °Cd^{-1}$) during exponential growth before canopy closure, conveyed by the ability to translate a given biomass gain into maximal new gain (Dingkuhn *et al.* 1999; Poorter 1999; Shipley 2006). Plant parameters contributing to RGR are leaf photosynthesis (source), assimilate partitioning among sinks (plant leaf-mass ratio in $g\ g^{-1}$), plant leaf area ratio ($m^2\ g^{-1}$ of plant), SLA ($cm^2\ g^{-1}$) and leaf appearance rate or leaf number (Poorter 1999; W. ter Steege *et al.* 2005).

Crop models simulate exponential growth as a result of a feedback loop between (1) light interception and conversion into assimilate, (2) partitioning of the new assimilate into new leaf biomass, and (3) converting this biomass into leaf area using SLA, resulting in increased interception (Brisson *et al.* 1998; Wang *et al.* 2002; Jones *et al.* 2003). This resource-capture driven model of vigor is extremely sensitive to SLA, a parameter that sets the metabolic cost of leaf area production and is considered genotypic (Dingkuhn *et al.* 1998; Dingkuhn *et al.* 1999) but in fact has low heritability

(Rebetzke *et al.* 1999; Rebetzke *et al.* 2004). This conceptual model does not take into account developmental dynamics of the plant, namely the rate of organogenesis (leaves, tillers) constituting the demand sides of the sink-source system.

Recently, (Dingkuhn *et al.* 2007) suggested that organogenetic processes and the resulting sink dynamics are a driving force for growth. (Luquet *et al.* 2006) presented a rice growth model (EcoMeristem) simulating growth-development interactions, namely competition for assimilates among sinks that feed back on both organogenesis and photosynthesis. According to this concept, early vigor should be strongly related to developmental rate (DR) i.e. leaf appearance rate (1/phyllochron). It is important to note that DR is defined here on an organogenetic basis (rate of phytomer production) and not earliness of flowering, two phenomena that are not necessarily linked.

Branching (tillering), organ size and organ abortion (senescence) would then have complementary effects to that of DR on vigor, or compensatory effects in the case of resource limitation. In addition, transitory carbohydrate reserves act as a buffer for sink-source imbalances in the case of excessive or limiting, aggregate sink capacity (Luquet *et al.* 2006; Dingkuhn *et al.* 2006, Legros *et al*. 2009).

According to this conceptual framework, high DR, or rapid organogenesis, favours an efficient conversion of assimilate gains into plant structure and thus acts as a pacemaker for growth. Across genotypes in a given environment, growth rate tends to be positively correlated with tillering ability (Goto *et al.* 1989; Yin *et al.* 1996; Dingkuhn *et al.* 1999; Miyamoto *et al.* 2004). Tillering controls the number of axillary buds that can potentially produce other tillers (Nemoto *et al.* 1995; Itoh *et al.* 1998; Miyamoto *et al.* 2004). As this would lead to excessive competition for resources within the plant (Dingkuhn *et al.* 2006; Luquet *et al.* 2006) followed by organ senescence and restriction of elongation processes, outgrowth of new tillers is regulated by apical dominance (Fetene *et al.* 1993; Bangerth *et al.* 2000). Such adjustments thus set a limit to growth rate and vigor (Granier *et al.* 1999; Luquet *et al.* 2005a; Dingkuhn *et al.* 2006). Consequently, organ number and size are generally negatively correlated across genotypes (W. ter Steege *et al.* 2005; Luquet *et al.* 2005a), but can be expected to be positively correlated across environments.

The compensatory relationship among the different components of early vigor necessarily involves strong genotype x environment interactions (GxE) on trait expression. This, as well as unknown physiological and/or genetic linkages among traits, makes the study and improvement of vigor traits complex (Itoh *et al.* 1998; Miyamoto *et al.* 2004; W. ter Steege *et al.* 2005). An important question is thus which of the components of vigor show wide genetic diversity that may be selected for (Poorter *et al.* 2000; Fuentes *et al.* 2005) and thus warrants genetic studies (Itoh *et al.* 1998; Li *et al.* 2003; Miyamoto *et al.* 2004; Morita *et al.* 2005; W. ter Steege *et al.* 2005).

The underlying hypothesis of the present study was that constitutive, genotypic DR is a driver of early vigor and is thus responsible for some of its phenotypic variation in rice. In the context of an ongoing association study, phenological and morphological information was generated independently on two panels. This paper is the first of a series of two articles. Its objective is to investigate the association of genotypic DR with early vigor and its relationship with other traits known to contribute to vigor such as tillering and leaf size. Subsequent papers will model early vigor using the conceptual framework provided here, and investigate the effectiveness of DR under drought.

MATERIAL AND METHODS

Experimental data

Two experimental data sets were generated in independent experiments (Exp1 and Exp2). In both experiments plants were grown during the vegetative phase under non-limiting nutrient and water conditions in a completely randomized design with three (Exp1) and two replicates (Exp2).

Data for Exp1 were obtained on Panel 1 (genetic composition further down) in the field from 18 March (sowing) to 24 April 2006 (end of destructive sampling at 35 days after sowing DAS). This fell into the dry season in the tropical environment of the upland research farm of the International Rice Research Institute (IRRI, Los Baños, the Philippines; 14°11'N, 121°15'E, 21m altitude). The field was not used under continuous cultivation and left fallow in alternating seasons, as during the wet and dry season of 2005.

Average air temperature during the experiment was 26.7°C with an average daily global radiation of 20.7MJ.m-**²**. The soil was a silty clay loam classified as Aquandic Epiaquoll, consisting of 47% silt, 35% clay and 18% sand, pH(H_2O) of 5.53, 1.27% organic C, and 0.12% total N. Plants were grown on freely drained soil after direct seeding at 5 grains per hill. Irrigation was provided twice a week to fill the soil reservoir to field capacity. Weeds were removed manually as necessary. Fertilizer was applied as a single basal application at a rate of 40 (N as urea), 30 (P as triple super phosphate and 30 (KCl). kg ha^{-1}. The experimental design consisted of 3 completely randomized blocks of 216 rows of 2 m length each, each row corresponding to one genotype. Three reference genotypes were repeated in 5 times per block. Rows were spaced at 25 cm with one hill every 10 cm (40 hills m^{-2}). In each plot 3 plants were tagged two weeks after sowing for subsequent, non destructive monitoring of plant development and growth (as described further down). At the same time the population was thinned to one plant per hill of an original population of up to 5 plants per hill. Daily air temperature, solar radiation and air humidity data were obtained from a standard agro-meteorological station on the farm.

The second data set was generated in a greenhouse experiment (Exp2) on Panel 2 (see details below) performed in 2009 at CIRAD (Montpellier, France) between 9 February and 8 May 2009 (late winter and early spring).

The greenhouse was S2 type (for GMO cultivation) equipped with a double glass roof intercepting much of natural sunlight. It was thus equipped with supplemental light sources (halogen lamps at 1.5 m spacing). Air humidity and temperature were regulated by adiabatic method and were set to 25°C/22°C (day/night) and 50%/90% air humidity. Seed was grown in a germination chamber at 29°C, then transplanted 3 days after germination in 1 litre drained pots (5 seeds per pot) when seedlings were about 3 cm tall. The date of transplanting was variable depending on the genotype and its time of germination. Pots were placed on flooded tables at 30 pots m^{-2} with 5 cm water depth. Plant populations were thinned to 1 plant per pot at 4-leaf stage. Pots contained about 450 g (dry weight) of a mixed soil consisting of 20% peat and 80% loamy sandy clay soil (loam: 45%, sand: 30% and clay: 25%, sampled at 0-40 cm depth in a field at Lavalette experimental site of Agro Montpellier, France), supplied on the day before transplanting with 2g of a coated fertilizer Basacote Plus 6M complemented in oligo-elements (Compo GmbH & Co. KG, Münster, Germany containing 11, 9 and 19% of N, P_20_5 and K_20 respectively). The experiment included a drought treatment that will be reported elsewhere and only well-watered treatment results are presented here. Because of high plant number and limited space the replications were implemented successively and not simultaneous, resulting in different radiation environments in the greenhouse.

Genetic materials

Panel 1 (169 O. *sativa* L. genotypes) was a subset of the rice core collection of the Generation Challenge Programme (GCP 2005). It consisted of 130 indica, 23 tropical japonica, 8 temperate japonica, 10 aus, 15 aromatic rices, 4 deep-water rices and 2 intermediate accessions obtained from the International Rice Genebank at IRRI.

Panel 2 (190 genotypes) consisted of 173 tropical japonica accessions composed to capture the genetic diversity of the sub-species, and an additional set of 17 reference accessions (extended Perlegen population) covering all genetic groups of the species (7 indica, 3 tropical japonica, 3 temperate japonica, 2 aus, 2 aromatic). The Perlegen population corresponds to the OryzaSNP panel currently used to develop a high-density SNP chip for genotyping (McNally *et al*. 2009).

Both panels were composed with the objective to conduct genetic association studies on drought tolerance and early vigor traits in the context of the Generation Challenge Programme (GCP).

Measurement of environmental variables

In both experiments environmental conditions were characterized for Photosynthetically Active Radiation (PAR) using quantum sensor SPK 215; global radiation (Rg) using pyranometer CS300; air temperature (T) using thermocouples (Campbell scientific, USA) and relative air humidity (RH) using humidity sensors (HMP 45C, Vaisala, Finland). Air humidity, Rg and PAR were measured at the top of the plants while T was characterized above (Exp2) and inside the canopy (Exp1) at an hourly time step. RH was measured at an hourly time step above the canopy.

T was used to compute thermal time (TT) as a sum of daily temperature (hourly integration) minus a base temperature of 12°C (Gao *et al.* 1992).

Plant measurements, data processing and normalization steps

In Exp1, plants were destructively sampled at 35 days after sowing for the three replications. In Exp2 plants were sampled at variable age depending on when the parallel drought treatment attained the targeted level of stress (not presented). In both experiments plants were characterized by the number of leaves appeared on the main stem (a leaf being considered as appeared when its tip emerges from the sheath of the previous leaf) to compute Haun Index, HI (Haun 1973), phyllochron and its reciprocal DR, (following Eq.3), tiller number (NBT, plant^{-1}), total leaf number (differentiating green leaves fully expanded, expanding leaves and dead leaves), and dimensions of the last ligulated (length of blade plus sheath, LL, and blade width LW). At the end of the experiment plants were sampled to measure shoot dry weight (SDW). All measurements, excepted shoot dry weight that was measured only at final sampling, were performed at two dates in each experiment (15 and 35 days after direct seeding in Exp1; when a genotype had 6 leaves appeared on the main stem and at final sampling at variable dates depending on genotypes in Exp2). In Exp2, the length of the first leaf on the main stem LL1 (blade + sheath, considering the first leaf with well differentiated blade and sheath) was measured at transplanting time; while in Exp1 LL1 was measured on seedlings grown in Petri dishes in an incubator, specifically to measure this trait.

Plants at sampling dates were characterized by developmental stage in terms of Haun Index and thermal time elapsed since sowing (Exp1) or germination (Exp2).

Considering a SDW of 0.001g one day after germination, the relative growth rate (RGR) of plant shoots was computed using the equation (Eq.1) provided by (Dingkuhn *et al.* 1998):

$$RGR = \left(SDWf / SDWi \right)^{1/TTif} \quad \text{in g g}^{-1}\,\text{°Cd}^{-1} \text{ (Eq.1)}$$

This equation expresses the change in dry matter between two dates (SDWi and SDWf), normalized by the current dry matter, under the assumption that relative gain per unit of thermal time is constant (exponential growth). The assumption of exponential growth is justified by the fact that populations were still far from canopy closure at the time of sampling.

In order to compare genotypes sampled at various dates and phenological ages (in terms of Haun Index) within Exp2 and between Exp1 and Exp2, growth related variables in Exp2 (SDW, tiller number NBT, total leaf number LN) were normalized by an environmental variable combining the incident daily radiation (PAR) and thermal time (TT) accumulated during plant growth. This photothermal variable (PT) was computed as (Eq.2):

$$PT = \left(\sum_n PAR\right) \times TT$$, in MJ °Cd m^{-2} (Eq.2)

Normalized growth variables of Exp2 were multiplied by the PT value of the plants in Exp1 at 35 days after sowing (i.e. 2.2×10^6 MJ °Cd m^{-2}).

Since in both experiments LL and LW were measured on leaves of various ranks, observations were normalized by this rank. Then, assuming linearity of change in leaf dimensions with rank during plant exponential growth (Dingkuhn *et al.* 2006; Luquet *et al.* 2006), i.e. until Rank 7, values were multiplied by 7 to estimate LL7 and LW7.

Developmental rate (inverse of phyllochron) was calculated using the thermal time accumulated from sowing (Exp1) and germination in the incubator (Exp2) to final sampling and the number of appeared leaves on the main stem (LN_MS) measured at the end of the experiment, following (Eq.3):

DR= (LN_MS)/ TT, in $°Cd^{-1}$ (Eq.3),

Statistical analyses

The Npar1way procedure of SAS for windows V.9.2 (Statistical Analysis System institute, Cary, NY, USA) was used to compare parameter distributions across genotypes using the Wilcoxon Mann Withney Test. Matrices of correlation were performed with the SAS Correlation procedure. XLSTAT software (2008 version 3.02, copyright Addinsoft 1995-2008; www.xlstat.com) was used to perform multiple linear regressions. Sigmaplot9 software (for windows version 9, copyright 2004, Systat software inc.) was used for plotting data and non-linear regressions.

RESULTS

Range of environmental conditions and phenotypic diversity

Figure 1 presents the distribution of thermal time and incoming PAR accumulated from germination to sampling for individual plants in each replication of Exp2. Large variations can be observed across genotypes within and among replications due to variation in transplanting and sampling dates. These results justify the normalization of data by thermal or photo-thermal variables as defined in Eq.1 and 2 in order to compare genotypes within and between experiments. Only normalized data will be considered in the following.

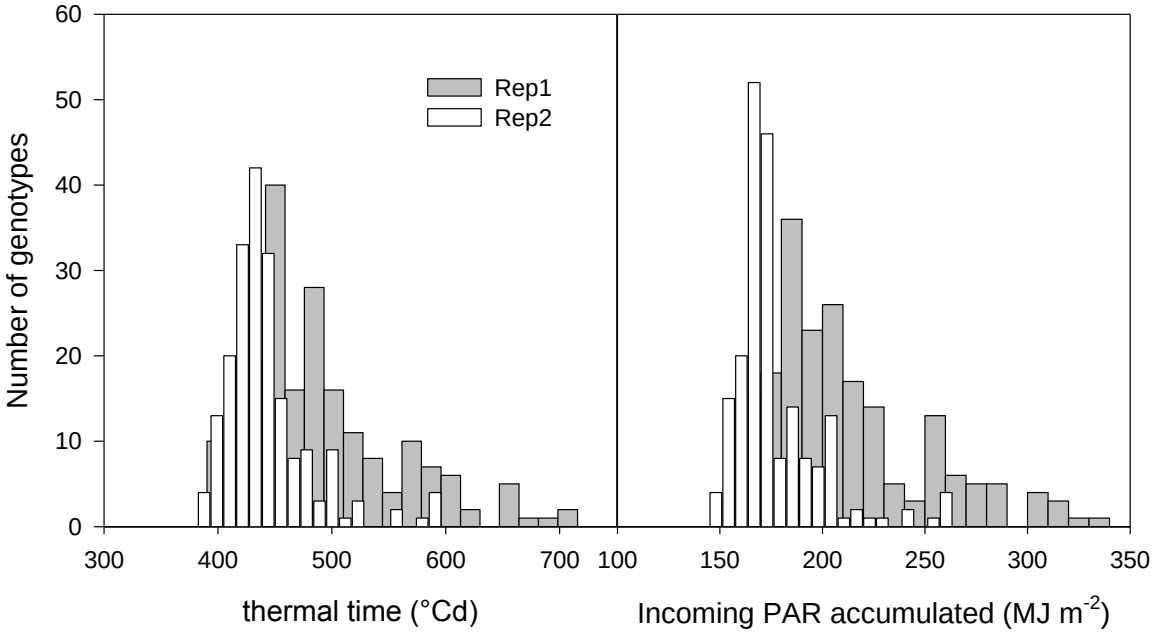

Figure 1: distribution of (a) thermal time and (b) incoming Photosynthetic Active Radiation (PAR) accumulated from plant germination to sampling in the two replications (Rep) of Exp2 for 190 genotypes.

In both experiments, normal distribution was observed within the panels for all variables observed **(Figure 2)**. The two-tailed Mann-Whitney rank sum test analysis for the two samples indicated a significant difference ($P < 0.001$) between experiments for all variables. Values were generally smaller in Exp1 than in Exp2. The causes are unknown but may be related to a combination of: i) population slightly denser in the field, ii) limited soil volume in pots restricting root growth to the advantage of shoot growth, iii) hydrology with a permanent soil saturation in pots and iv) genotype differences between experiments with mainly indica in Exp1 and japonica in Exp2. The difference was small for tiller number (**Figure 2a**) but large for leaf dimensions (**Figure 2ef**), normalized SDW (**Figure**

2b) and RGR (**Figure 2b**), where distributions for the two experiments did not overlap at all. Developmental rate (DR, $°Cd^{-1}$) was intermediate with distributions distinctly different but overlapping (**Figure 2c**).

Numerical differences observed among genotypes for RGR were expressed as the factor by which aboveground biomass changed per unit degree-day. The observed range between 1.011 and 1.016 for Exp2 corresponds to 1.1 and 1.6 % net growth per degree-day, or ca. 15% to 23% per day. The range of 0.014 to 0.022 $°Cd^{-1}$ observed for DR in Exp2 corresponds to a phyllochron of 71 to 45 °Cd or about 5.1 to 3.2 days under the experimental conditions.

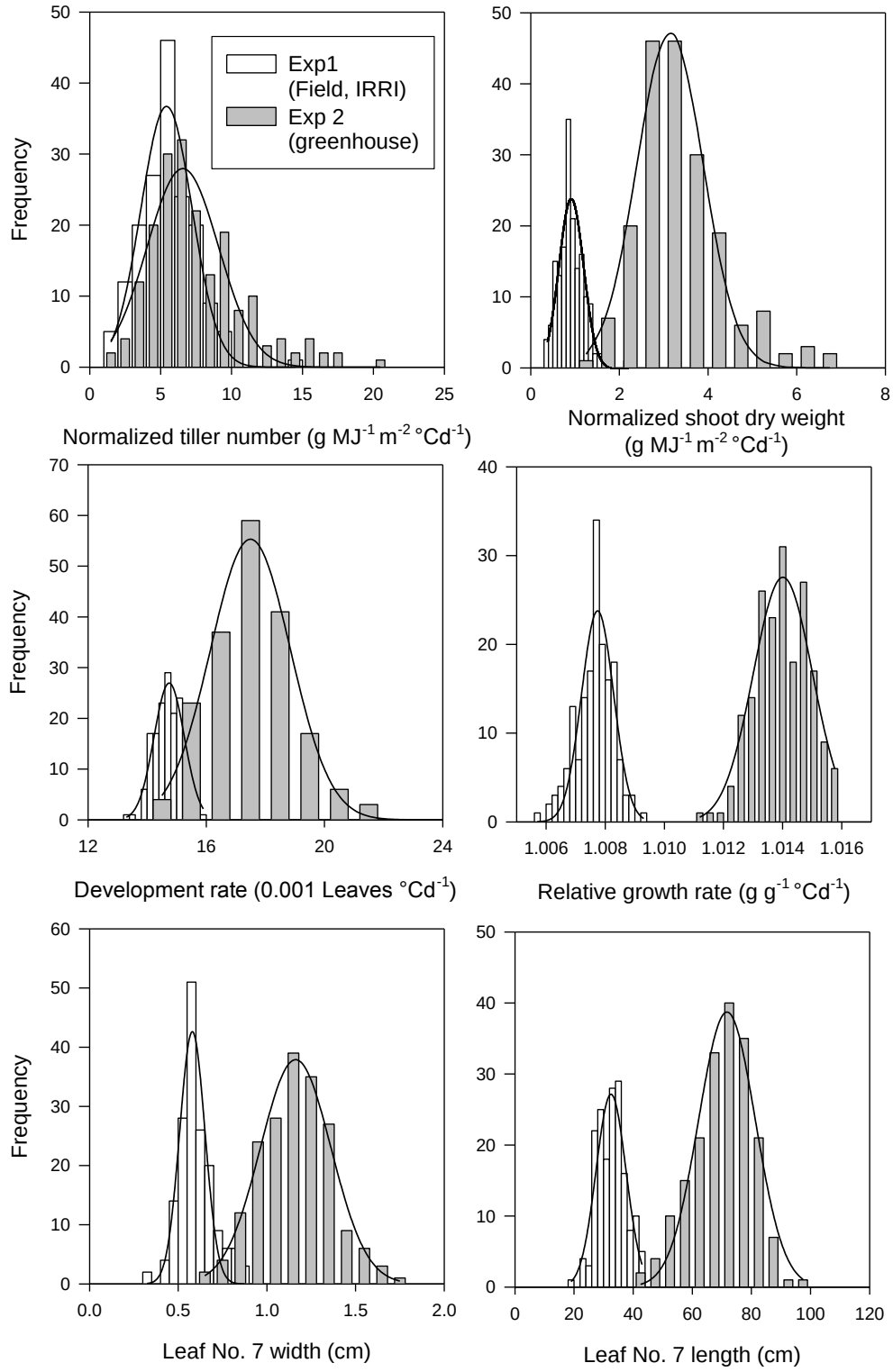

Figure 2: *Frequency distribution in Exp1 and Exp2 of variables normalized for a photothermal accumulation PT of 2.2x10^{6} MJ. °Cd.m^{-2}: plant tiller number, plant shoot dry weight, estimated final width and length of leaf 7 on the main stem, developmental rate and relative growth rate (RGR, g.g^{-1}.°Cd^{-1}). Average values on 3 and 2 replicates respectively in Exp1 and Exp2.*

Correlations among traits across genotypes

Correlations among observed variables across genotypes showed similar patterns for Exp1 (Table 1) and Exp2 (Table 2). R values had the same orientation between experiments for all pairs of variables and were significant (P<0.01) for the same pairs of variables in most cases. Negative but weak correlations were observed for leaf size (width and length) vs. tiller number and DR, suggesting that genotypes with large leaves produce less tiller and develop more slowly. Stronger, positive correlations were observed between SDW DR and tiller number in both experiments. In Exp1, the strongest correlation with R>0.6 was observed between tiller number and dry weight related variables (RGR and SDW). The strongest correlation in Exp2 was between DR and RGR (R=0.69), although tiller number vs. RGR and DR were only slightly less correlated. The high correlation between SDW and RGR is of no significance because these two variables were not independently measured.

In both experiments, LL1 was positively (P<0.01) correlated to RGR and SDW. It was not significantly correlated to DR in Exp1 and negatively (P<0.01) in Exp2.

According to multiple linear regression analyses, the variables DR, number of tillers and leaf length explained significantly (P< 0.0001) genotypic variation in RGR by 56 % in Exp1 **(Table 3)** and to 67 % in Exp2 **(Table 4)**. The number of tillers had the greatest partial contribution to variation of RGR in Exp1 (T = 10.2), whereas DR had the greatest contribution in Exp2 (T = 12.0).

Table 1: Matrix of correlation (Pearson correlation coefficients, N = 169) between variables measured at 35 days after sowing or calculated between sowing and 35 days after sowing (DR, RGR) in Exp1. SDW, shoot dry weight in g; RGR, shoot relative growth rate (g g-1Cd-1); DR, developmental rate (°C d-1); LW7, LL7 respectively estimated width and length of leaf 7 on the main stem and NBT, tiller number. Bold values indicate highly significant correlations (p<0.01). * for P < 0.05; ** for P < 0.01.

Variables	LL7	LW7	NBT	DR	RGR
LW7	**0.226****	1			
NBT	-0.021	**-0.271****	1		
DR	-0.030	**-0.207****	**0.356****	1	
RGR	**0.342****	0.010	**0.631****	**0.421****	1
SDW	**0.331****	0.057	**0.658****	**0.430****	**0.942****

Table 2: Matrix of correlation (Pearson correlation coefficients, N = 190) between variables measured at plant sampling or calculated between germination and sampling (DR, RGR) in Exp2 in the two replications. SDW (g): Shoot dry weight estimated for a photothermal variable, PT, of 2.2x10^{6} MJ m^{-2} °Cd; RGR, shoot relative growth rate (g g^{-1} Cd^{-1}) DR, developmental rate (°Cd^{-1}); NBT, tiller number estimated for a PT of 2.2x10^{6} MJ^{-1} m^{2} °Cd^{-1}; LW7 and LL7 respectively main stem leaf 7 final width and length. Bold values indicate highly significant correlations (p< 0.01). * for P < 0.05; ** for P < 0.01.

Variables	LL7	LW7	NBT	DR	RGR
LW7	**0.474****	1			
NBT	**-0.193****	**-0.240****	1		
DR	**-0.356****	**-0.411****	**0.572****	1	
RGR	0.158*	0.077	**0.595****	**0.688****	1
SDW	**0.202****	0.175*	**0.527****	**0.247****	**0.540****

Table 3: Multiple linear regression model explaining relative growth rate (RGR, g g^{-1} °Cd^{-1}) in Exp1 (field experiment).

Nb: Inflation factor was computed and inferior to 2 for all the variables, validating the stability of the regression model. A ridge regression was also performed, confirming the same r² values, the significance for each parameter and for the model (p<0.0001) and finally the higher effect of tiller number (PROC REG Ridge procedure of SAS software).

RGR = 1.00 + 0.278 DR + 1.800 E-4 NBT + 3.309 E-4 LL7 (Eq.3)

Adjusted R^2 = 0.565

N = 169

Predictor variable	Absolute Coefficient	Normalized Coefficient	Normalized Std	T	P
Intercept	1.001			1045.7	< 0.0001
DR	0.278	0.234	0.054	4.303	< 0.0001
NBT	1.800E-4	0.555	0.054	10.203	< 0.0001
LL7	3.309E-4	0.361	0.051	7.095	< 0.0001

Table 4: Multiple linear regression model explaining relative growth rate (RGR, g g^{-1} °Cd^{-1}) in Exp2 (greenhouse experiment)

Nb: Inflation factor was computed and inferior to 2 for all the variables, validating the stability of the regression model. A ridge regression was also performed, confirming the same r^2 values, the significance for each parameter and for the model (p<0.0001) and finally the higher effect of DR (PROC REG Ridge procedure of SAS software).

RGR = 1.00 + 0.457 DR + 1.863 E-4 NBT + 2.924 LL7 (Eq.4)

Adjusted R^2 = 0.674

N = 190

Predictor variable	Absolute Coefficient	Normalized Coefficient	Normalized Std	T	P
Intercept	1.003			1453.07	< 0.0001
DR	0.457	0.648	0.054	12.008	< 0.0001
NBT	1.863E-4	0.309	0.051	6.075	< 0.0001
LL7	2.924E-4	0.462	0.045	10.296	< 0.0001

Illustration of the direct relationship between developmental rate and growth

A much stronger correlation was observed in Exp2 than in Exp1 for DR vs. RGR. This may be due: i) to the differences in genotypes used in these experiments and ii) to the same environmental constraints in the field that led to smaller organ size and plant dry matter as mentioned above. These constraints being absent in Exp2 (greenhouse), we sought to further analyze this relationship under such potential conditions.

Scattergrams with values for both replications for SDW and RGR vs. DR are shown in **Figure 3**. Particularly for RGR vs. DR, a close correlation was observed. Given the fact that effects of DR on growth may be either direct (through the higher frequency of leaf appearance, thus accelerating leaf area development during exponential growth) or indirect (e.g., through greater tiller production), we sought to eliminate the effect of tiller number and leaf size from the growth (SDW or DR) vs. RGR relationship. Groups of observations were extracted from the data that have common leaf length (LL7 of 72.5 cm +/- 2.5cm range, including sheath) and three classes of tiller number (2.4 +/- 0.25, 4.7+/- 0.85 and 8.7 +/- 0.85). Tiller number was given particular attention here because potential tillering is directly related to DR through number of tiller buds. It is thus important to determine

whether DR affected RGR through tillering or also independently of tillering. Leaf length effects on RGR were at the same time excluded by choosing similar genotypes in this respect. Within these subsets of genotypes, the positive correlation between growth and DR was conserved, and it was similar among the classes. Consequently, DR had a positive effect on SDW and in particular on RGR independently of tiller number and leaf size. This was supported by multiple regressions, inflation factor and ridge regression analyses shown in **Table 4**, confirming a significant, independent effect of DR, as well as tiller number and leaf size on RGR. The same analysis performed on Exp1 gave similar results but at a lower level of correlation (data not presented).

Interestingly, for two genotypes (Azucena, a tropical japonica and IR64, an indica) present both in Exp1 and Exp2, DR during vegetative growth was near-identical for Azucena in both environments, with 0.0139°Cd^{-1} +/- 0.0008 in Exp1 vs. 0.0140°Cd^{-1} (+/- 0.0029) in Exp2. IR64 showed greater variability of DR between experiments, with 0.0148°Cd^{-1} (+/- 0.0007) in Exp1 vs. 0.0188°Cd^{-1} (+/- 0.0012) in Exp2 but it always had higher values than Azucena ($P<0.05$). The same trend was observed in terms of RGR (g g^{-1} °Cd^{-1}) with, for Azucena, 0.01393 (+/- 0.00084) in Exp1 and 0.01399 (+/- 0.00295) and, for IR64, 0.014805 +/- 0.00070 in Exp1 and 0.018749 +/- 0.00070 in Exp2. DR and RGR were thus heritable in the broad sense but were also influenced by environment, in terms of the different conditions in Exp1 and Exp2.

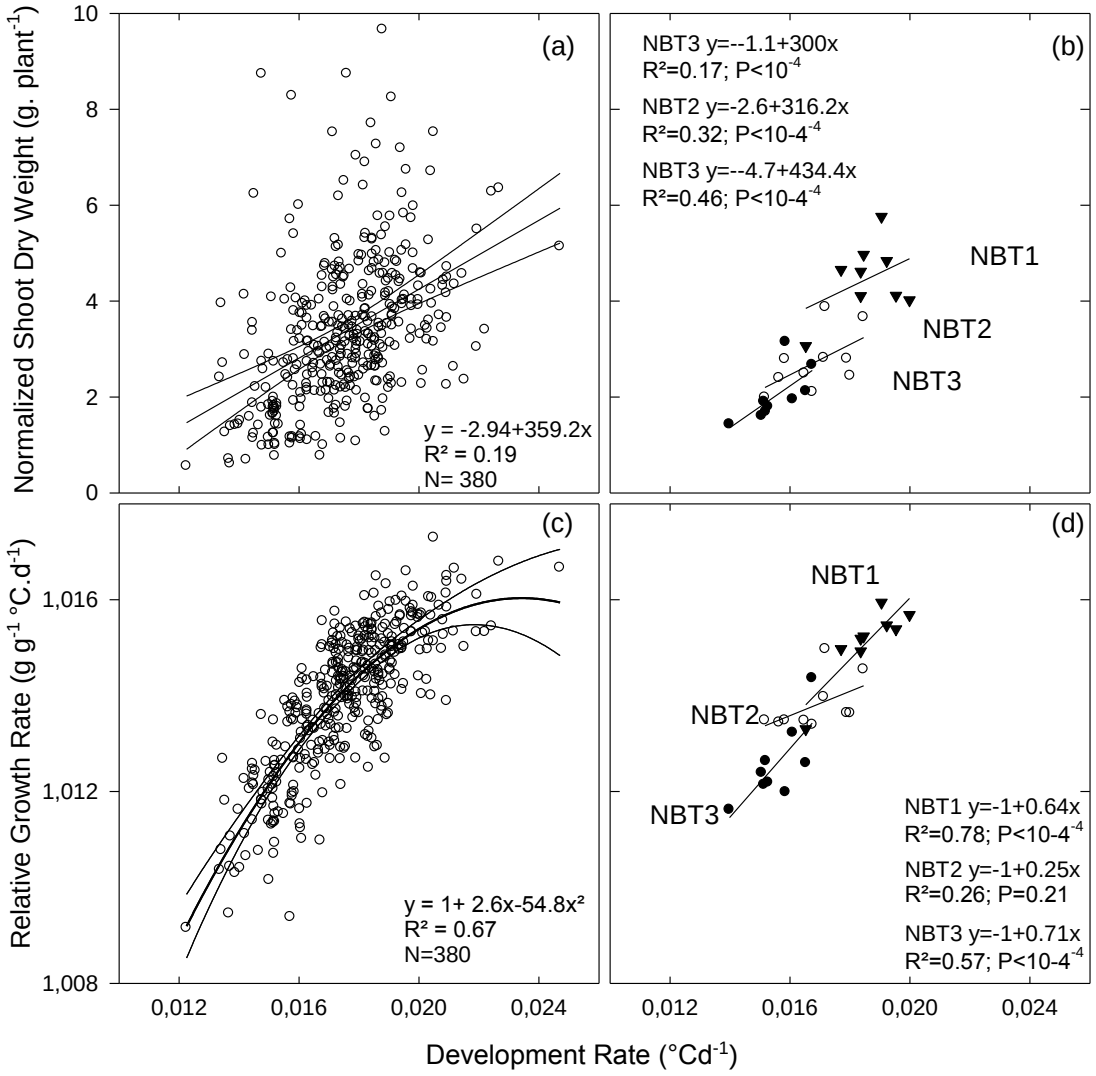

***Figure 3**: Relationship between developmental rate DR and (a, b) shoot dry weight estimated for a PT (photothermal variable) of 2.2x106 MJ.m-².°C.d and (c, d) shoot relative growth rate: (a, c): N = 380 and (b, d): in three subsets of plants sharing the same leaf size (leaf 7 length of 72.5 cm +/- 2.5, including blade and sheath) and each defined by similar tiller number, NBT: NBT1: 2.4 tillers +/- 0.25, N=11, P<10-4 in (b, d); NBT2: 4.7 tillers +/- 0.85, N=11, P<10-4 in (b) and P=0.21 in (d); NBT3: 8.7 tillers +/- 0.85, N=11, P<10-4 in (b, d)). On individual plants of the two replicates in Exp2 (2009 greenhouse experiment, Montpellier, France). In (a, c) regression curves are associated with confidence interval at P=0.01.*

DISCUSSION

We tested the hypothesis that DR, which is the pace-maker of plant organogenesis (Itoh *et al.* 1998; Miyamoto *et al.* 2004; Miyoshi *et al.* 2004), is also a major driver of early vigor in rice. Observations on DR and several component traits of early vigor (SDW, RGR, leaf size and tillering) conducted on

two different rice diversity panels during exponential growth indicated a highly significant correlation between DR and dry weight growth parameters, notably RGR which is known as a measure of early vigor (Poorter 1999). This correlation was stronger in Exp2 on japonica panel than in Exp1 on indica panel. The data do not permit attributing this difference to panel composition or to environment.

Early vigor is not a simple trait and can be conveyed, in a given resource situation and environment, by several component traits that are physiologically interacting and may involve environment dependent trade-offs (W. ter Steege *et al.* 2005). It is thus difficult to isolate the effect of a single component trait of early vigor. In fact, in the present study tiller number and leaf size also contributed to dry weight accumulation, in addition or in interaction with DR. Evidence for DR *per se* having a positive effect on vigor was in this study derived from the observation that within subsets of genotypes having similar leaf length and tiller number, the effect of DR on vigor was conserved and not weaker than for the entire panel. This result is important and suggests that DR per se is a potential key trait to breed for improved vigor.

Rice genotypes having rapid development thus tend to have higher RGR and therefore develop ground cover and biomass more rapidly during exponential growth, at least under non-limiting resources when their potential can be expressed. This effect is due to the more rapid succession of phytomer and leaf production (higher DR values). In turn, it accelerates the production of tiller buds associated with the phytomers, and therefore potentially accelerates tillering (Miyamoto *et al.* 2004). In fact, DR and tiller production were consistently positively correlated in this study, as previously demonstrated by other authors (Rebetzke *et al.* 1999; Morita *et al.* 2005; W. ter Steege *et al.* 2005). The negative correlation observed between leaf size and both tiller number and DR is probably the result of a physiological trade-off or compensation as commonly observed in dwarfing, which in rice involves smaller leaves but higher tiller number (Zou *et al.* 2005).

Strong evidence for the physiological linkage of DR (in terms of plastochron) and tillering was found in mutants. For example, in recent studies on rice mutants having modified plastochron (Itoh *et al.* 1998; Asai *et al.* 2002; Miyoshi *et al.* 2004) or tillering (Komatsu *et al.* 2003; Zou *et al.* 2005), the physiological linkage between tillering and DR was not overcome and the phenotype of the mutants was systematically modified on both traits when compared to the wild type. Similar evidence on physiological trait linkages (based on the genetic modification of a single gene) was also shown for leaf size vs. DR (Itoh *et al.* 1998; Miyoshi *et al.* 2004): rice mutants affected on a gene controlling the rate of leaf primordium initiation systematically presented reduced leaf size compared to the wild type. In the present study, an important result is that this trade-off can be overcome, which makes it

possible to breed for superior early vigor through high DR while maintaining high number of tillers and leaf size.

Nevertheless, genetic linkages among these traits may be involved. While no genetic linkage was found among tillering and DR in previous studies on rice (Miyamoto *et al.* 2004). W. ter Steege *et al.* (2005) suggested on wheat that the apparent physiological linkages among traits constituting early vigor at different scales (cell, leaf, whole plant) could have genetic bases. Tisné *et al.* (2008) suggested the same for *Arabidopsis thaliana,* the orientation of the trait linkages being dependent on the alleles present on different QTLs involved. These recent findings emphasize that the genetic study of traits involved in plant morphogenesis and plasticity should not be addressed by looking at a single trait (Granier *et al.* 2009). The data presented here for two rice diversity panels will provide answers once the genomes of the populations have been densely genotyped and association studies conducted for the traits phenotyped.

While in Exp1 (field) tillering made a greater contribution than DR to biomass accumulation, it was the opposite in Exp2 (greenhouse, japonica panel). Leaf size had contributed less than tillering or DR. This confirms previous studies showing a stronger effect on plant growth of organ number (leaf or tiller) compared to organ size (Rebetzke *et al.* 1999; Morita *et al.* 2005; W. ter Steege *et al.* 2005).

Size does matter, however, as shown by (Rebetzke *et al.* 1999; Rebetzke *et al.* 2007) for coleoptile length in wheat seedlings having different vigor. In our study DR and first leaf length LL1 were negatively and significantly correlated ($p<0.01$ for Exp 2). However, LL1 was positively ($p<0.01$ for Exp1 and Exp2) correlated with SDW suggesting an important positive effect of initial leaf length on growth. There may thus be a trade-off between DR and size of early leaves on vigor.

Several QTLs were found for DR in various cereals but this trait is known to be prone to GxE. This raises the question of its heritability (Miyamoto *et al.* 2004; Morita *et al.* 2005; Borràs-Geloncha *et al.* 2010). In this study, two contrasting rice cultivars, Azucena and IR64, were part of Exp1 and Exp2 and IR64 showed significantly higher DR compared to Azucena. Similar differences in DR were also reported for the two varieties by Ahmadi *et al.* (2008) and Luquet *et al.* (2005) based on different experiments. This confirms that DR is a heritable trait in the broad sense but further studies are required to characterize its specific heritability through crosses, as well as its interaction with environment.

Next steps for the present work will be the study of the genetic control of DR, as well as its interaction with other traits in the whole plant system and its effects on vigor in sub-optimal environments such as drought (Granier and Tardieu 2009; Tisné *et al.* 2008).

CONCLUSIONS

This study tested the hypothesis that DR is a major driver of early vigor in rice, and that genotypic differences in vigor should therefore correlate with it across panels representing genetic diversity. The hypothesis was fully confirmed under greenhouse conditions for a population representing the diversity of the tropical japonica subtype of *O. sativa*. Trends were similar and significant too for a species-wide panel phenotyped in the field but correlations were weaker for DR, whereas tillering ability showed a stronger effect on growth vigor. Importantly, the effects of DR were in both cases significant within sub-groups having similar tillering activity and leaf size, traits frequently correlated with DR. The results therefore suggest that improvement of DR through breeding is worth exploring, despite physiological linkages and trade-offs between tillering potential, organ size. Heritability of DR is considerable as indicated by conservation of varietal differences across environments, but needs more study.

More research is also needed on the genetic and physiological basis of genotypic differences in DR, as well as genotype x environment interactions on the effect of DR on early growth vigor. Whole-plant modelling with adequate representation of crop growth vs. phenology process interactions should lay a theoretical basis for understanding how DR affects growth vigor and interacts with environment and other traits. This will be the objective of the sequel to this paper.

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Headlines in Chapter I

- Recent works suggested that sink dynamics may be a driving force of resource acquisition and thus, early vigour defined by shoot biomass accumulation.

- An experimental (this chapter) and modelling approach (article in Annex) were combined to explore whether sink dynamics, defined by DR (Development Rate, 1/phyllochron), tillering and leaf size, are main drivers of early vigor in rice plants.

- Sink related traits showed a wide variation both within the tropical japonica panel (190 genotypes) studied in controlled conditions and the O. sativa panel studied in field conditions (201 genotypes).

- Shoot biomass accumulation was positively related to DR, tiller number and leaf size. DR was positively correlated to tiller number, and both traits were negatively correlated to leaf size.

- To overcome any artefact due to the physiological linkage between DR, tillering and leaf size, the correlation between SDW and DR was confirmed considering three subsets of 10-15 genotypes with the same leaf size and tillering ability.

- The modeling study (Apendix I) confirmed these linkages and suggested that DR was the main driver of early growth, before light conversion efficiency. Also it suggested that high DR, vigorous seedlings have the propensity to rapidly use C assimilates for growth, by contrast with sink limited plants that store starch. This was experimentally confirmed by a negative correlation between plant growth rate and starch concentration measured in source leaves on a subpanel of 43 japonica genotypes.

- Modeling results also suggested the need to explore to which extent traits related to C source acquisition can explain early vigor diversity.

- The relationships between sink related traits constituting early vigor and the way the plant uses C assimilates, under both well watered and drought conditions must be further explored. This will be addressed in the next chapter.

Chapter II : Phenomics of rice early vigour and drought response: Are sugar related and morphogenetic traits relevant?

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ABSTRACT

Early vigour is a useful but complex trait in rainfed rice (*Oryza sativa* L). Little is known on the impact of vigour traits under drought. This study investigates on 43 genotypes to which extent early vigour under drought is related to genotypic differences in morphogenetic and metabolomic traits. A greenhouse experiment was conducted to characterize plant morphogenesis and sugar concentration in expanded (source) and expanding (sink) leaves.

In control treatment, leaf starch concentration was negatively correlated with organogenetic developmental rate and positively with leaf size. Under drought, vigour was associated with stronger growth reduction. Starch concentration decreased in source leaves, by contrast with soluble sugars and with that observed in sink leaves.

Accordingly, genotypes were grouped in three clusters differing in constitutive vigour, starch storage and growth maintenance under drought. Results are discussed regarding novel process based traits to be introduced in the GRiSP (Global Rice Science Partnership) phenotyping network.

Keywords: sativa rice; early vigour; drought regulation; developmental rate; leaf size; non structural carbohydrates

INTRODUCTION

Rice production is limited by water availability in rainfed rice (*Oryza sativa* L.) ecosystems. Progress in developing improved, drought adapted cultivars has been slow during the last decades. Understanding plant diversity is relevant to assess plant behaviour in relation to adaptation to drought-prone environments (Alonso-Blanco *et al.* 2009). Originating from flood-prone ecosystems frequently exposed to drought, where it was domesticated, rice was selected for various rainfed environments including dryland (upland) and rainfed lowland ecosystems. This resulted in large genetic and phenotypic diversity, of high value today to breeding for drought tolerance and yield potential traits (Ni *et al.* 2002).

Drought is particularly harmful to rice during the crop establishment phase and at flowering, requiring different physiological adaptations: stomatal conductance (Farooq *et al.* 2010), osmotic adjustment (Fukai *et al.* 1995), hormonal or metabolic regulations (Wang *et al.* 2006; Luquet *et al.* 2008; Fumagalli *et al.* 2009), root and shoot morphogenesis (Price *et al.* 1999; Liu *et al.* 2004; Asch *et al.* 2005; Karaba *et al.* 2007; Luquet *et al.* 2008), and organogenesis (Schlosser *et al.* 2000; Yan *et al.* 2005; Jahn *et al.* 2011) are reported as mechanisms conferring drought tolerance in targeted environments. During early vegetative growth crop stand is established, tillers are formed and organs for resource capture (leaf canopy and root system) are deployed. These processes also affect resources available during later crop development phases (Finch-Savage *et al.* 2010), for example through delays of flowering and maturity that can extend the growth cycle into the dry season (Wopereis *et al.* 1996). During the vegetative phase rapid ground cover achieved with early vigour (Dingkuhn *et al.* 1999; Poorter *et al.* 1999; Shipley 2006; Finch-Savage *et al.* 2010) can reduce soil evaporation, accelerate root access to soil water and nitrogen, and reduce competition with weeds (Zhao *et al.* 2006c). Early vigour may also accelerate depletion of soil water reserves, making less water available for later crop stages (Zhang *et al.* 2005). However in aerobic rice culture with fluctuating soil moisture early vigour is associated with yield stability (Okami *et al.* 2011).

High relative growth rate (RGR, $g.g^{-1}.°Cd^{-1}$) during exponential growth before canopy closure, conveyed by the plant's ability to translate a given biomass gain into maximal new gain commonly defines early vigour (Dingkuhn *et al.* 1999; Poorter *et al.* 1999; Shipley 2006). Early vigour depends on both assimilate source (light capture and photosynthetic rate) and the sink constituted by structural

growth (leaf appearance rate, potential size and tiller outgrowth). A recent study conducted under non-limiting resources (Rebolledo *et al.* in press ,Luquet *et al.* in press) identified organogenetic developmental rate (DR: 1/phyllochron), together with tillering ability and leaf size, as major genotypic determinant of rice early vigour. The results suggested trade-offs between organ number and size. Across a large number of genotypes, DR was positively correlated with tillering and negatively with leaf size and leaf starch concentration. The authors hypothesised that the lower starch concentrations observed in leaves of vigorous, high-DR genotypes reflect source-limited behaviour caused by strong internal demand for assimilates. Component traits of early vigour are thus in part physiologically linked in terms of trade-offs, but may also be linked genetically (ter Steege *et al.* 2005; Granier *et al.* 2009).

Physiologically linked traits frequently occurring together can be seen as an expression of adaptation strategies. Phenotyping and genetic analyses of traits linked by compensatory processes or co-selection are more meaningful if applied to the functional ensemble of traits (Aguirrezabal *et al.* 2006). Simultaneous use of metabolic markers as non structural carbohydrates (NSC) and growth related variables can help to understand plant adaptation strategies and identify relevant traits (Stitt *et al.* 2010). For rice crop improvement, Ishimaru *et al* (2007) suggested to consider carbohydrate metabolites together with physiological traits. This is thought to be particularly relevant for vegetative stage drought (Cabuslay *et al.* 1999; Jahn *et al.* 2011).

Under drought, both structural growth (sink) and assimilation (source) processes are down regulated, resulting in changed source-sink relations that may depend on environment and genotype. Plant passes from a carbon (C) source to sink limited situation as the reduction of organ growth and development (i.e. sink activity) appears to happen earlier than C starvation under water deficit conditions (Muller *et al.* 2011). Previous data (Luquet *et al.* 2008) showed that drought causes a decrease in source leaf starch concentration, whereas in sink leaves and the apex, starch and sucrose are accumulated in rice seedlings. The latter is associated with an increase in cell wall invertase activity but a decrease in hexose concentration. Thus, under drought, apex tissues actively import C but use it more for reserve accumulation than for growth. Observations on roots were similar. Other studies demonstrated that sugars act as both metabolic resource and signal under water stress, participating in the regulation of organ growth and development (Liu *et al.* 2004; Rolland *et al.* 2006; Stitt *et al.* 2007; Ramel *et al.* 2009). Accordingly, NSC are intrinsically related to early vigour and its maintenance under drought. This raises the question whether plant phenomics research, in the quest for efficient molecular breeding tools for drought tolerance, should consider metabolic markers such as sugars.

For breeders, component traits directly or indirectly contributing to yield (Campos *et al.* 2004) are useful if they are easy to measure and correlated with yield, while having greater genetic diversity than yield itself (Tuberosa *et al.* 2002). Phenotyping for molecular breeding purposes allows developing molecular probes for marker-based selection. In this context, it is important that markers for component traits of a complex trait have proven physiological complementarities (synergies) while being under distinct genetic control.

The overall objective of the present study was to explore morphogenetic and metabolic traits of rice related to early vigour and its maintenance under water limited conditions. Specific objectives were to (i) identify constitutive and response traits associated with vigour and drought tolerance, (ii) compare whether these rice genotypes differed in traits related to the morphogenetic process and primary C metabolites. The study was conducted on vegetative plants of 43 genotypes, composed mainly of tropical japonica upland rices. Perspectives for phenomics and research on adaptation strategies are discussed on the basis of the results, in particular in the context of the GRiSP (Global Rice Science Partnership) research programme phenotyping network of the CGIAR (Consultative Group of International Agricultural Research).

MATERIALS AND METHODS

Genetic material

A collection of seeds of 202 rice genotypes were received from different locations in Asia, America, Africa and Europe. This collection contains 178 tropical japonica, 17 indica, 3 temperate japonica, 2 Aus and 2 aromatic. Morphogenetic relations under well watered conditions were previously reported on (Rebolledo *et al.* in press). An initial subset of 43 genotypes was randomly selected for metabolic analysis. This subset includes 31 tropical japonicas, 11 indica, 3 temperate japonica, 2 Aus and 2 aromatic rices, according to the isozyme based classification of (Glaszmann *et al.* 1984). Only data on this subset **(Table 1)** will be addressed in this study.

Table 1 Description of the 43 genotypes studied. Genetic groups defined according to (Glaszmann et al. 1984) (Tjap : Tropical Japonica, Indica, Aus, TempJap: Temperate Japonica and Aro: Aromatic cultivars), Seed origin, general classification (Improved: I or Traditional: T) and classification according to clusters defined in this study (Clust)

Germplasm name	Sub-specie	Origin	Clust
APO	indica	Philippines	1
ASD 1	indica	India	1
Azucena	tjap	Philippines	2
Basmati 370	aro	India	1
Bico branco	tjap	Brazil	1
Bulu pandak	tjap	Indonesia	3
Cha phu ma	tjap	Thailand	2
Cirad 394	tjap	Madagascar	1
Cirad 488	tjap	Madagascar	1
CT13582-15-5-M	tjap	Colombia	3
Cuba 65	tjap	Cuba	2
Dom sofid	aro	Iran	3
Dourado agulha	tjap	Brazil	2
Dourado precoce	tjap	Brazil	2
Early mutant iac 165	tjap	Brazil	3
Fandrapotsy 104	indica	Madagascar	2
Fossa hv	indica	Burkina fasso	1
Gotak gatik	indica	Indonesia	1
IAC 165	indica	Brazil	2
IAC 25	tjap	Brazil	2
IR64	indica	Philippines	1
IR71525-19-1-1	tjap	Philippines	3
IR72967-12-2-3	indica	Philippines	3
Jao haw	tjap	Thailand	3
Kendinga5h	tjap	Malaysia	3
Ketan meh	tjap	Indonesia	2
Khao dam	indica	Laos	2
Kindang patong	tjap	Philippines	3
M 202	tempjap	USA	2
Maintimolotsy 1226	tjap	Madagascar	3
Moroberekan	tjap	Guinea	2
N22	aus	India	1
OS 6	tjap	Zaire	2
Padi rotan	indica	Indonesia	3
Padiboenar	tjap	Indonesia	3
Peh pi nuo	tjap	China	2
Pratao	indica	Brazil	1
Primavera	tjap	Brazil	1
Reketmaun	tjap	Indonesia	3
Tequing	indica	China	1
Vietnam2	tjap	Vietnam	1
Vietnam3	tjap	Vietnam	3
Yunlu 7	tjap	China	2

Plant culture

A greenhouse pot experiment was performed in 2009 at CIRAD (Montpellier, France) between 9 February and 8 May 2009 (late winter and early spring).

The greenhouse was S2 type (for GMO cultivation) with a double glass roof intercepting much of natural sunlight. It was thus equipped with supplemental light sources (halogen lamps at 1.5 m spacing). Mean radiation was 4.6 $MJ.m^{-2}$. Air humidity and temperature were regulated by adiabatic method and were set to 25°C/22°C (day/night) and 50%/90% air humidity. Seeds were grown in a germination chamber at 29°C. When seedlings reached 3 cm height, 5 seedlings per pot were transplanted in 1 litre drained pots (see Rebolledo *et al.* (in press) for details). The date of transplanting was variable depending on the genotype and its time of germination. Pots were placed on flooded tables with 5 cm water depth. Plants were thinned to 1 plant per pot at 4-leaf stage. Within a replication each genotype was represented by two potted plants, one for the well watered treatment and one for the drought treatment. Pots contained about 450g (dry weight) of a mixed soil consisting of 20% peat and 80% loamy sandy clay soil (loam: 45%, sand: 30% and clay: 25%, sampled at 0-40 cm depth in a field at Lavalette experimental site of Agro Montpellier, France). The mixed soil was characterized by a field capacity of 59% and a wilting point of 11% moisture content (mass/mass on the basis of dry weight), supplied with 2g of a coated fertilizer Basacote Plus6M complemented in oligo-elements (Compo GmbH & Co. KG, Münster, Germany containing 11, 9 and 19% of N, P_20_5 and K_20, respectively).

Water deficit treatment

When the plant of a given genotype, tagged for future water stress application, reached the stage of 6 leaves appeared on the main stem (6-leaf stage), water treatments were differentiated. This stage, together with pot size, was chosen to eliminate the expression of drought avoidance through root distribution effects, while permitting the expression of varietal differences in tolerance. Plants in the well watered treatment were kept on a shallowly flooded table. Water stressed plants were initially irrigated from the top of the pot up to saturation, and then drained to achieve field capacity. The soil surface of the pot and the drainage holes were then covered with a plastic film to avoid any water loss by evaporation, any subsequent weight loss being caused by plant transpiration during dry-down. Pots were weighed twice a day, in the morning (from 7 to 9 am) and in the evening from (4 to 6pm). Pot weight was used to calculate the Fraction of Transpirable Soil Water FTSW (noted here FTSW', Eq.1, by contrast with FTSW of Luquet *et al* (2008) since FTSW' considers the same WP value for all genotypes). The dry down system was detailed by Luquet *et al* (2008).

$$FTSW' = \frac{AW - WP}{FC - WP} \quad \text{(Eq.1)}$$

In Eq.1, AW is the actual weight of a given pot; WP and FC are respectively corresponding pot weights at wilting point and field capacity.

The relation between FTSW' and predawn leaf water potential on excised ligulated leaves was realized in the same growing conditions with one genotype. A FTSW' value of 0.2 (± 0.05) corresponded to leaf water potential of -0,8MPa.

Sugar Analysis

At FTSW' 0.2, the two last ligulated leaves (youngest source leaves) and the expanding leaves (sink leaves, enclosed in the sheath of oldest leave) were sampled in the morning (before 10 am) to analyse NSC content: hexoses (glucose and fructose), sucrose and starch. Sugar content was analysed based on High Performance Liquid Chromatography (HPLC; see Luquet *et al.(* 2006) for details). The results are expressed in mg of glucose per g of dry matter (mgGLU.g^{-1}) for starch and in mg of sugar per g of dry matter (mg.g^{-1}) for hexoses and sucrose. Hexoses concentrations were considered equal to glucose plus fructose concentrations. Sugar related variables are named combining the type of organ sampled (sink: SINK or source: SOUR) and the type of sugar (HEX: hexoses; SUC: sucrose and STA:starch). For example the concentration of sucrose in source leaves is named SOURSUC.

Growth measurements

All genotypes were sampled and measured at the same drought level when FTSW' value reached 0.2 (+/- 0.05), which occurred at different dates among genotypes.

Table 2 summarises measured variables at the end of the treatment, which included tillers per plant (NBT), total ligulated leaf number (NBL, corresponding to ligulated green plus senescent leaves on the plant), and dimensions of the last ligulated leaf (length LL and width LW). Plants were then sampled to measure shoot dry weight (SDW), adding the dry weight of the leaves collected for sugar content analyses.

The number of leaves on the main stem was used to compute Haun Index, HI (Haun 1973).On its basis, the mean phyllochron and its reciprocal Developmental Rate, (°C.d^{-1}) were calculated from germination until the end of the treatment (DR). DR_{onset} corresponded to the phyllochron computed from the period of stress onset to the end of stress , both for stressed (d) and its corresponding well watered plant(c).

Variables were indexed as c (for control well watered plants) and d (for plants under water deficit) for example for shoot dry weight SDW_C and SDW_d.

In order to compare genotypes transplanted and sampled at different dates morphogenetic variables and final biomass were normalized by a photo-thermal variable (PT), combining the incident daily radiation (PAR) and thermal time (TT) accumulated during plant growth. This photothermal variable (PT) was computed as (Eq.2):

$$PT = \left(\sum_n PAR\right) \times TT \text{ , in MJ.°C.d.m}^{-2} \text{ (Eq.2)}$$

Leaf area of the last ligulated leaf on the main stem was computed using LL, LW and an empirical allometric coefficient of 0.725 (Tivet *et al.* 2001). Considering leaf size increase with rank is linear during the exponential growth phase (Dingkuhn *et al.* 2006), leaf size was normalized by its rank in order to compare varieties following Eq.3;

$$\text{LDIM} = (\text{LL}*\text{LW}*0.725)/ \text{ leaf rank (Eq.3)}$$

Senescence was estimated for both stressed and well watered plants. For each individual ligulated leaf on the main stem the senescence was visually quantified as the percentage of dead tissue vs. total leaf area (%senescleaf), then to take into account differences of plant age, the percentage of senescence was normalized by HI as in Eq. 4:

$$\text{LSEN} = \frac{\sum(\%senescleaf)}{HI} \text{ (Eq.4)}$$

Leaf rolling (ROL) was estimated using SES (IRRI 1996) for the whole plant for both treatments.

Computation of drought response variables

Variables measured at the end of the treatment on well watered plants were considered as constitutive variables. Drought response variables were calculated using Eq. 5, applied to both morphological and sugar related variables:

$$\text{VAR}dc/c = \frac{(\text{d - c})}{(\text{c})} \text{ (Eq.5)}$$

In Eq.5 d and c are the values of a given variable under drought and well watered conditions respectively. According to this, a negative value of $VAR_{dc/c}$ corresponds to a reduction by the drought treatment.

Table 2 Summary of morphogenetic and metabolic variables and units performed in this study (dw: dry weight, PT: photothermal time Eq. 3)

Variables		Unit	Measured variables at the end of the experiment
morphogenetic	DR	leaves .°C d-1	Leaf formation rate from germination date to final sampling
	Dronset	leaves .°C d-1	Leaf formation rate from stress onset to final sampling
	LDIM	cm 2 .leaf rank	Last ligulated leaf area on the main stem normalized by leaf rank
	NBT	tillers. PT	Total tiller number on the plant normalized by PT
	NBL	ligulated leaves. PT	Total ligulated leaf number on plantnormalized by PT
	SDW	g. PT	Shoot Dry weight normalized by PT
	LSEN	% death tissue	Percentage of death leaf tissue of ligulated leaves on the main stem
	ROL	rolling index	Rolling index estimated with SES (IRRI 1996)
metabolic	SINKHEX	mg.g-1 dry weight	Metabolite concentration in the hidden, expanding (sink) leaf of the main stem
	SINKSTA	mgGLU.g-1 dry weight	
	SINKSUC	mg.g-1 dry weight	
	SOURHEX	mg.g-1 dry weight	Metabolite concentration in the two last ligulated (source) leaves of the main stem
	SOURSTA	mgGLU.g-1 dry weight	
	SOURSUC	mg.g-1 dry weight	

Data analysis

Statistical analysis was performed with statistical software R (http://www.R-project.org).

Anova model with multiple factors (genotype, treatment and replication) was used to estimate the part of variance related to the genotype and the treatment. Comparison of means was performed using Tuckey test and correlations were performed using spearman correlation coefficients. The FactoMiner Package in R was used for multivariate analysis (Lê *et al.* 2008). To introduce several variables simultaneously as active elements a multiple factorial analysis (MFA) was used. MFA works as a Principal Component Analysis, the variables being weighted (Husson *et al.* 2010), and this avoids the dominance of variables having the largest variance in the construction of the first axis. The objective of this analysis is to identify the main axes representing data variability.

To translate MFA results into a typology (varietal clusters) we used the methodology developed by (Husson *et al.* 2011) to consolidate groups of genotypes with a simultaneous analysis of a principal component map, followed by hierarchical and aggregative clustering. Indeed, all 26 variables underwent MFA, constituting its principal components. Then, 9 dimensions were selected because they give the best partition quality (minimum of Intracluster/Intercluster distance ratio) and because these 9 dimensions together accounted for 81% of total variability.

RESULTS

Morphogenetic and metabolic variables under well-watered conditions

A MFA was performed among variables measured under well watered conditions **(Figure 1a)**. The first two axes explained 48% of total variance observed. Both axes were positively related to SDWc and SOURSUCc **(Figure 1a)** and separated variables in three groups: (i) variables related to organ number: NBTc, NBLc, DRc (positively related to the first axis), (ii) one variable related to organ size (LDIMc) and variables related to NSC: starch and hexoses in source and sink leaves and sucrose in sink organs (positively related to the second axis), (iii) variables related to organ senescence (LSENc) and constitutive leaf rolling (ROLc), which showed opposite response to SDWc on the second axis **(Figure 1a)**.

In terms of genotype distribution on axes (not shown), both axes separated genotypes according to SDW. Some genotypes had similar SDW but different traits were associated with it: IR64, an indica check, had high organ number, low SOURSTAc and SINKSTAc while Dourado agulha, a japonica type, had big leaves and high non structural carbon (NSC) concentrations.

Linear correlations among variables across genotypes under well watered conditions were analyzed by spearman correlation matrix **(Table 3)**. SDWc was positively and significantly ($p<0.01$) correlated with organ number (NBTc and NBLc) and leaf appearance rate (DRc). The effect of LDIMc on SDWc was positive but not highly significant ($p<0.1$). Variables related to organ number, however, were significantly ($p <0.01$) and negatively correlated to LDIMc.

Leaf number (NBLc) and DRc were negatively correlated with SOURSTAc ($p<0.01$), and positively correlated with SOURSUCc ($p<0.05$). LDIMc was positively correlated with SOURSTAc, SINKSTAc ($p<0.05$) and SOURHEXc ($p<0.01$) **(Table 3)**.

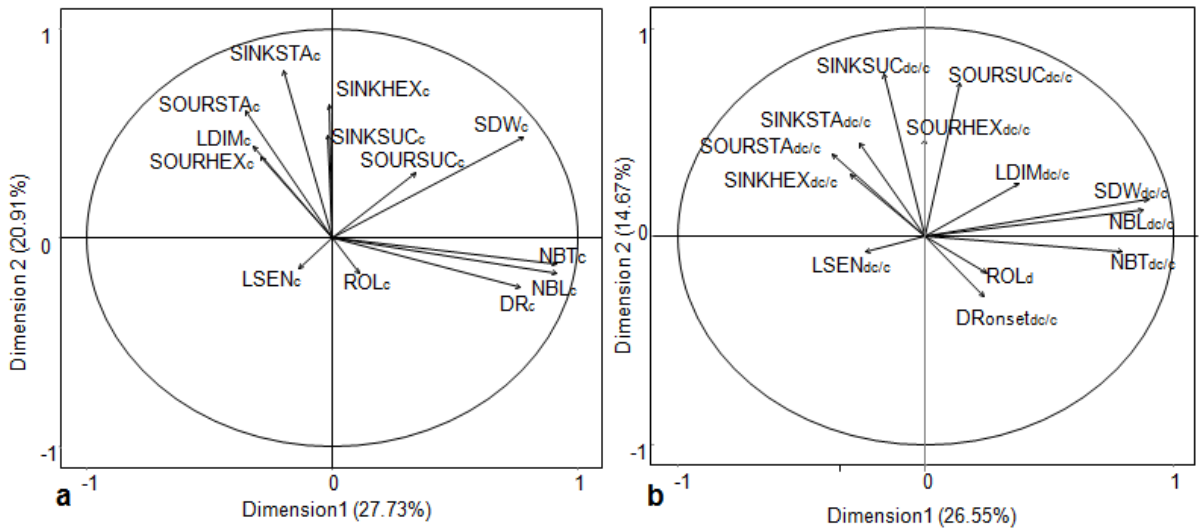

Figure 1 *Factorial plans with the two principal components representing morphogenetic and metabolic (sugar) variables averaged on two replications for 43 rice genotypes under well watered conditions (a) and in response to water limited conditions (b). Variance explained by each dimension is shown as a percentage of total variance (indicated in axis legend). Each variable is represented by a vector connecting the origin to the variable coordinates. Coordinates correspond to the correlation coefficients between variables and dimensions 1 and 2*

Table 3 Spearman correlation matrix for average morphogenetic and metabolic variables measured at the end of the experiment under well watered conditions, considering 43 varieties, 2 replicates; significance levels are indicated with ***p<0.01, **p<0.05

	SINKSTAc	SOURSUCc	SINKSUCc	SOURHEXc	SINKHEXc	LDIMc	DRc	NBLc	NBTc	LSENc	ROLc	SDWc
SOURSTAc	**0.52*****	0.23	0.21	0.17	0.29	**0.35****	**-0.37****	**-0.39*****	-0.24	-0.04	-0.08	0.01
SINKSTAc		0.08	**0.47*****	0.16	**0.41*****	**0.42*****	-0.12	-0.2	-0.16	-0.21	-0.05	0.22
SOURSUCc			0.22	0.02	0.00	-0.03	**0.29****	**0.32****	**0.31****	**-0.33****	-0.29	0.27
SINKSUCc				0.11	**0.38*****	-0.07	0.02	-0.06	-0.04	0.02	-0.25	0.06
SOURHEXc					-0.03	**0.33****	-0.24	**-0.34****	**-0.35****	0.04	**0.32****	0.06
SINKHEXc						0.05	-0.14	-0.04	0.01	0.07	-0.12	0.20
LDIMc							**-0.43*****	-0.29	-0.22	-0.08	0.21	0.14
DRc								**0.78*****	**0.67*****	-0.06	0.07	**0.39*****
NBLc									**0.92*****	-0.16	0.05	**0.57*****
NBTc										-0.19	0.04	**0.64*****
LSENc											0.28	-0.08
ROLc												0.19

Effect of water deficit on morphogenetic and metabolic variables

Drought reduced significantly ($p<0.01$) SDW by 18.8%, and LDIM by 12.4%. SOURHEX and SINKHEX were significantly ($p<0.01$) increased **(Table 4)**. LDIM, SOURHEX, SINKHEX, showed increased genotypic variability under drought **(Table 4)**. SOURSTA was reduced by 54.36%, ($p<0.01$), and genotypic variance was reduced under drought. Stressed plants showed significantly higher LSEN and ROL than well-watered plants **(Table 4).**

The difference between treatments for SOURSUC and SINKSTA was not significant **(Table 4)**. Reduced genotypic variation was observed under drought for variables related to organ number (NBT, NBL, DRonset) and starch (SOURSTA and SINKSTA). Although treatment effect was not significant for NBT, NBL was significantly ($p<0.05$) reduced for stressed plants (Table 4).

Table 4 Average, standard deviation (sd), minimum (min) and maximum (max) values for variables calculated under well watered and drought conditions. Averages on 43 varieties and 2 replicates for morphogenetic and metabolic variables. Stress effect is noted dc/c (corresponding response to drought of the variable on the same line in the table) and expresses the relative variation from stress to control plant. P-values ***<0.001; **<0.01; *<0.05, ·<0.1, ns, no significant; ANOVA results are presented with respective significances for G (Genotype) or T (Treatment) effects.

Variables		Well watered treatment				Drought Treatment				Stress effect dc/c	Anova
		Average	Sd	Min	Max	Average	Sd	Min	Max		
Morphogenetic	Dronset	1.43E-02	3.33E-03	7,99E-03	1,66E-02	1.35E-02	3.10E-03	8,30E-03	2,00E-02	-0,06	G*, T.
	LSEN	0.34	0.128	0,15	0,64	0.389	0.157	0,14	0,56	0,12	G** ,T**
	ROL	0.18	0.59	0	3	4.18	2.28	0	9	0,96	G*, T***
	SDW	1.56E-05	6.52E-06	7,80E-06	1,68E-05	1.26E-05	4.49E-06	4,84E-06	2,43E-05	-0,19	G ns ,T***
	NBT	4.85E-05	2.82E-05	2,18E-05	8,54E-05	4.72E-05	2.65E-05	1,55E-05	1,06E-04	-0,03	G***, Tns
	NBL	5.11	1.68	1,81	6,74	4.485	1.457	2,23	8,23	-0,12	G*** ,T**
	LDIM	1.41E-04	5.92E-05	7,67E-05	2,22E-04	1.35E-04	5.74E-05	7,91E-05	2,84E-04	-0,04	G*** ,Tns
Metabolic	SOURHEX	13.42	7.53	5,49	32,36	33.55	16.17	1,72	14,48	1,50	G*** ,T***
	SOURSUC	78.77	19.76	46,05	110,27	77.69	20.93	37,1	109,61	-0,01	G**, Tns
	SOURSTA	48.10	50.32	0,36	97,17	21.96	35.38	5,01	124,11	-0,54	Gns, T***
	SINKHEX	78.92	44.05	27,2	89,97	102.85	36.92	5,84	74,09	0,30	G*, T***
	SINKSUC	76.30	24.28	53,7	134,04	100.31	24.60	23,83	119,73	0,31	G ns ,T***
	SINKSTA	79.95	31.30	26,42	129,62	74.87	31.87	36,62	128,24	-0,06	G*** ,Tns

Relations between constitutive traits and vigour maintenance under stress

Correlations between constitutive variables (observed in control well watered plants, **Table 5**) and drought response variables (Eq.5) were performed. SDWc was negatively ($p<0.01$) correlated with the response of biomass under drought(SDWdc/c), leaf appearance rate during the treatment (DRonsetdc/c), last ligulated leaf dimensions (LDIMdc/c) and tillering (NBTdc/c). Consequently, plants producing high SDW when well watered had greater relative reductions in SDW, organ number and organ size.

LDIMc was negatively ($p<0.01$) correlated with SOURSUCdc/c and SOURSTAdc/c. Then, plants having large leaves had a strong decrease in SOURSUC and SOURSTA under drought. By contrast, DRc was positively ($p<0.05$) correlated with SOURSUCdc/c and SOURSTAdc/c **(Table 5)**.

Table 5 Partial spearman correlation matrix among morphogenetic or metabolic variables measured at the end of the experiment under well watered conditions and calculated response variables. Average for each of the 43 varieties with 2 replicates; ***p<0.001, **p<0.05.

		Well watered morphogenetic variables					Well watered metabolic variables			
		SDWc	NBTc	LDIMc	NBLc	DRc	SOURSUCc	SOURSTAc	SINKHEXc	SOURHEXc
Response morphogenetic variables	$Dronset_{dc/c}$	**-0.38*****	**-0.46*****	**0.34****	**-0.53*****	**-0.61*****	-0.28	0.24	0.02	0.25
	$LSEN_{dc/c}$	0.18	0.06	0.09	0.04	-0.04	0.10	0.03	0.05	-0.09
	$LDIM_{dc/c}$	**-0.53*****	-0.18	**-0.40*****	-0.20	-0.16	0.03	0.15	-0.20	-0.22
	$NBT_{dc/c}$	**-0.42*****	**-0.48*****	-0.01	**-0.42*****	-0.15	-0.22	0.22	-0.12	0.20
	$NBL_{dc/c}$	**-0.49*****	**-0.35****	-0.13	**-0.35****	-0.09	-0.17	0.22	-0.07	0.08
	$SDW_{dc/c}$	**-0.59*****	-0.20	-0.20	-0.17	-0.05	-0.08	0.11	-0.27	-0.08
Response metabolic variables	$SOURSUC_{dc/c}$	-0.08	0.24	**-0.51*****	0.23	**0.32****	-0.28	-0.08	-0.02	**-0.43*****
	$SOURSTA_{dc/c}$	0.01	0.30	**-0.32****	**0.32****	**0.39****	0.05	**-0.48*****	-0.15	-0.29
	$SOURHEX_{dc/c}$	0.07	0.20	0.05	0.22	0.13	0.07	0.10	-0.18	**-0.62*****
	$SINKSUC_{dc/c}$	-0.01	0.12	-0.16	0.15	0.18	-0.12	-0.20	-0.26	**-0.33****
	$SINKSTA_{dc/c}$	-0.01	0.05	-0.23	0.09	0.04	-0.04	**-0.51*****	-0.29	-0.01
	$SINKHEX_{dc/c}$	0.09	0.03	0.22	0.02	0.10	-0.03	-0.11	**-0.59*****	-0.03

Correlations among drought response variables

Figure 1b presents the two first principal components (explaining 41% of total variation) of a MFA performed on drought response variables (dc/c). All morphogenetic response variables had a significantly positive effect on the first axis whereas the senescence response (LSENdc/c) had a negative effect. Consequently, drought effects on organ production rates were negatively correlated with drought effects on senescence.

NSC response variables participated significantly in the second axis, notably SOURSUCdc/c and SINKSUCdc/c (**Figure 1b**). The distribution of genotypes in the factorial plane (not shown) indicates that IR64 and Dourado Aghula had opposite behaviour but not on the first axis, suggesting similar morphogenetic response to drought but contrasting NSC response to drought.

Spearman correlation analysis on drought response variables (Table 6) confirmed the positive correlation between maintenance of SDW and maintenance of leaf size and organ number ($p<0.001$); but only $p<0.1$ for DRonsetdc/c. By contrast with observations under well watered conditions, drought response of organ number related variables were positively correlated with drought response of leaf size (LDIMdc/c). Consequently, although leaf number and size were constitutively opposed traits (under well watered conditions), their maintenance under drought was positively correlated.

SOURSTAdc/c was negatively ($p<0.05$) related to NBTdc/c and SOURSUCdc/c. There were no significant correlations between the response to drought of other morphogenetic and soluble sugar related variables.

Table 6 Partial Spearman correlation matrix among morphogenetic and metabolic response variables; average calculated for each of the 43 varieties with 2 replicates ***p<0.001, **p<0.05

	LDIM$_{dc/c}$	NBT$_{dc/c}$	NBL$_{dc/c}$	SDW$_{dc/c}$	LSEN$_{dc/c}$	ROL$_{d}$	SOURSUC$_{dc/c}$	SOURSTA$_{dc/c}$	SOURHEX$_{dc/c}$	SINKSUC$_{dc/c}$	SINKSTA$_{dc/c}$	SINKHEX$_{dc/c}$
Dronset$_{dc/c}$	0.06	**0.31****	0.25	0.20	-0.15	0.10	-0.18	-0.18	-0.24	-0.29	-0.15	-0.07
LDIM$_{dc/c}$		0.25	**0.48*****	**0.72*****	-0.02	0.15	0.27	-0.10	0.11	0.21	-0.04	0.00
NBT$_{dc/c}$			**0.76*****	**0.63*****	-0.02	0.19	-0.02	**-0.39*****	-0.04	-0.23	**-0.34****	-0.04
NBL$_{dc/c}$				**0.76*****	-0.15	-0.02	0.14	-0.13	-0.06	-0.13	**-0.35****	-0.08
SDW$_{dc/c}$					-0.25	0.14	0.14	-0.10	0.09	0.03	-0.24	0.02
LSEN$_{dc/c}$						-0.15	-0.07	-0.13	-0.03	0.01	0.01	0.10
ROL$_{d}$							-0.13	**-0.31****	0.15	0.03	-0.26	0.06
SOURSUC$_{dc/c}$								**0.36*****	0.15	0.42	0.04	-0.06
SOURSTA$_{dc/c}$									0.00	0.21	0.24	0.12
SOURHEX$_{dc/c}$										0.22	-0.17	**0.38****
SINKSUC$_{dc/c}$											**0.42*****	0.25
SINKSTA$_{dc/c}$												0.06

Genotype clustering based on constitutive and response traits

All studied variables were analysed by MFA followed by a clustering analysis. Three clusters were identified **(Figure 2)** and observed plant variables contributing significantly (P<0.001) to axes 1 and 2 in the previously described analyses (Table 7) were used to describe different adaptation strategies in the panel studied.

The first axis was composed of (i) drought effect variables (dc/c) with positive correlation coefficients and (ii) constitutive(c) organ number related variables with negative correlation coefficients **(Figure 2)**. Interestingly SOURSTAc was significantly (p<0.01) positive correlated to the first axis while SOURSTAdc/c was significantly (p<0.01) negative correlated to the first axis. The second axis was positively described by metabolic response variables SOURSTAdc/c, SINKSTAdc/c, SOURSUCdc/c and SINKSUCdc/c, while negatively described by control variables as LDIMc, SINKHEXc and SINKSTAc(p<0.001). This axis was also positively related (p<0.01) to drought effects on SDW (**Figure2**).

The highest nodes of the hierarchical clustering were represented in the factorial plane and defined the centres of gravity for the genotypes in each cluster **(Figure 2)**. Cluster 3 had positive coordinates on the first two axes opposite to Clusters 1 and 2. Cluster 1 had positive coordinates on axis 2 by contrast with Cluster 2 **(Figure 2)**. Consequently SDW, SOURSUC, SOURSTA, SINKSUC and SINKSTA of Cluster 3 showed a low response to drought, while drought response and control values of SOURSUC, SOURSTA, SINKSUC and SINKSTA differed between Cluster 1 and 2.

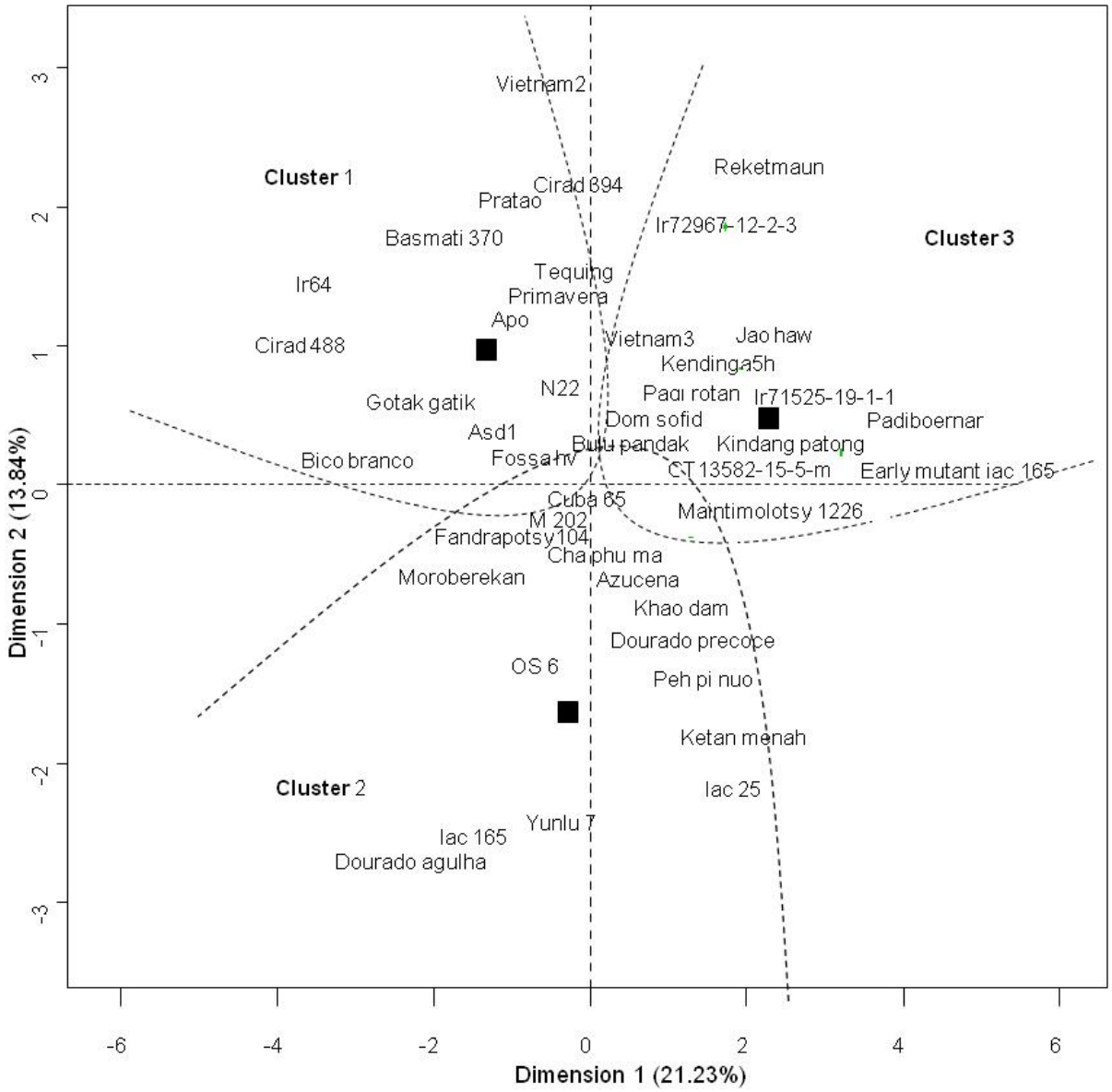

Figure 2 *Simultaneous representations of principal component map and hierarchical genotype clustering. Factorial plan is represented with the first two dimensions of nine, defined based on average variables (detailed in Table 2) on two replicates for each of the 43 rice genotypes studied Dimension 1 is significantly ($p<0.01$) and positively correlated to (in decreasing effect order): SDWdc/c, NBLdc/c, NBTdc/c, DRonsetdc/c, and SOURSTAc and negatively to: SDWc, NBLc, NBTc, DRonsetc, SOURSTAdc/c, SINKHEXdc/c. Dimension 2 is significantly ($p<0.01$) and positively correlated to (in decreasing effect order): SOURSUCdc/c, SINKSUCdc/c, SINKSTAdc/c, DRonsetdc/c, SOURSTAdc/c, SDWdc/c and negatively to: SINKSTAc, SINKHEXc, LDIMc, SOURHEXc, SOURSTAc, SINKSUCc. Resulting genotype clusters are delimited with dashed lines. Black squares represent the centre of gravity for each cluster (i.e. highest nodes of the hierarchical clustering). Since clusters were defined based on nine dimensions, two varieties close together can be in the same cluster or not (if they differ on other dimensions apart from 1 and 2).*

Similar numbers of varieties were assigned to each cluster: 14, 15 and 14 respectively in groups 1, 2 and 3 **(Figure 2,** Table 1 and Table 7**)**. The analyses presented above were based on morphogenetic observations normalized for differences in photo-thermal time because genotypes did not attain the 6-leaf stage synchronously. In **Figure 3** are presented absolute observations for morphogenetic variables as means for genotype clusters measured at the end of the drought and well watered treatments.

Addressing constitutive differences among clusters (well watered conditions) **Table 7 and Figure 2** show that Clusters 1 and 2 had both greater SDWc than Cluster 3 ($P<0.05$). These two vigorous groups differed among each other ($P<0.05$) in leaf and tiller number (greater in Cluster 1) and leaf size (larger in Cluster 2). The large leaves of Cluster 2 had almost twice the starch concentration of Cluster 1 ($P<0.05$, Table 7), Cluster 3 being intermediate.

Response variables within genotype clusters **(Figure 3)** show that, leaf size and plant height were significantly ($P<0.05$) reduced by drought in Clusters 1 and 2 but not in Cluster 3. Relation within each group between NBTdc/c and SOURSTAdc/c was also studied (not shown), for Cluster 1 and 2 SOURSTAdc/c was negatively related to NBTdc/c ($R^2=0.28$, $p<0.01$ and $R^2=0.19$, $p<0.01$ respectively). The relations in Cluster 3 showed a no significant positive ($p=0.3$, $R^2=0.08$) relation. NBT was only reduced in Cluster 1 **(Figure 1,** Table 7**)**, which also showed a significant decrease in source-leaf starch concentration despite the low constitutive level.

Consequently, Cluster 3 was more tolerant to drought than Clusters 1 and 2 under these experimental conditions.

Table 7 Summary of the clustering analysis with average values and standard deviations (sd) for each variable in each cluster. Multiple comparisons of means between groups and treatments at 95% confidence level (Tuckey test) are presented by letters. When there is a significant difference, the sign represents the sense of the difference (ns:no significant). Genotypes distant from the centre of gravity for each cluster are considered as the most representative of this cluster

	Cluster 1	**Sd**		**Cluster 2**	**Sd**		**Cluster 3**	**Sd**	
Genotypes distant from the center	IR64, Cirad 488, Bicobranco			Azucena, IAC 25, Dourado Aguila			Early mutant IAC 165, DomSofid, IR729679		
Number of individuals	14			15			14		
	Constitutive variables measure of early vigor								
SDW_c	1.80E-05	3.35E-06	**a** $^{+}$	1.71E-05	2.95E-06	**a** $^{+}$	1.24E-05	1.71E-06	**b** $^{-}$
$LDIM_c$	4.62	1.08	**b** $^{-}$	6.21	0.96	**a** $^{+}$	4.78	1.04	**b** $^{-}$
DR_c	1.65E-02	1.21E-03	**b** $^{+}$	1.50E-02	1.04E-03	**a** $^{-}$	1.55E-02	1.44E-03	**ab**
NBT_c	6.30E-05	1.88E-05	**b** $^{+}$	4.19E-05	1.01E-05	**a** $^{-}$	4.01E-05	1.45E-05	**a** $^{-}$
NBL_c	1.73E-04	4.21E-05	**b** $^{+}$	1.26E-04	2.19E-05	**a** $^{-}$	1.23E-04	2.71E-05	**a** $^{-}$
$SOURSTA_c$	33.46	22.75	**b** $^{-}$	61.48	24.01	**a** $^{+}$	55.43	24.14	**ab** ^{ns}
$SOURSUC_c$	78.44	10.38	**a** ^{ns}	81.71	14.08	**a** ^{ns}	79.84	12.22	**a** ^{ns}
$SOURHEX_c$	10.66	3.98	**b** $^{-}$	16.40	6.70	**a** $^{+}$	13.75	4.71	**ab** ^{ns}
$SINKHEX_c$	68.75	33.39	**a** ^{ns}	92.70	23.56	**a** ^{ns}	76.24	25.65	**a** ^{ns}
$SINKSTA_c$	69.90	23.22	**b** $^{-}$	96.02	18.68	**a** $^{+}$	75.18	19.13	**b** $^{-}$
$SINKSUC_c$	65.88	18.00	**b** $^{-}$	85.50	12.56	**a** $^{+}$	77.44	15.40	**ab** ^{ns}
ROL_c	0.29	0.52	**a** ^{ns}	0.23	0.40	**a** ^{ns}	0.07	0.17	**a** ^{ns}
$LSEN_c$	0.35	0.10	**a** ^{ns}	0.33	0.09	**a** ^{ns}	0.33	0.07	**a** ^{ns}
	Drought Response variables								
$SDW_{dc/c}$	-0.19	0.19	a $^{-}$	-0.24	0.20	**a** $^{-}$	0.18	0.25	**b** $^{+}$
$LDIM_{dc/c}$	-0.11	0.16	**ab** ^{ns}	-0.22	0.14	**a** $^{-}$	0.23	0.64	**b** $^{+}$
$Dronset_{dc/c}$	-0.13	0.16	**b** $^{-}$	0.07	0.19	**a** $^{+}$	0.00	0.12	**ab** ^{ns}
$NBT_{dc/c}$	-0.11	0.22	**a** $^{-}$	0.02	0.32	**a** $^{-}$	0.47	0.53	**b** $^{+}$
$NBL_{dc/c}$	-0.09	0.18	**a** $^{-}$	-0.09	0.16	**a** $^{-}$	0.15	0.16	**b** $^{+}$
$SOURSTA_{dc/c}$	-0.36	0.51	**b** $^{+}$	-0.76	0.23	**a** $^{-}$	-0.69	0.21	**a** $^{-}$
$SOURSUC_{dc/c}$	0.11	0.19	**b** $^{+}$	-0.15	0.12	**a** $^{-}$	0.04	0.22	**b** $^{+}$
$SINKSUC_{dc/c}$	0.79	0.61	**b** $^{+}$	0.18	0.22	**a** $^{-}$	0.38	0.29	**a-**
ROL_d	3.71	2.02	**a** ^{ns}	4.67	1.71	**a** ^{ns}	4.11	1.53	**a** ^{ns}
$LSEN_{dc/c}$	0.29	0.42	**a** ^{ns}	0.26	0.40	**a** ^{ns}	0.23	0.34	**a** ^{ns}

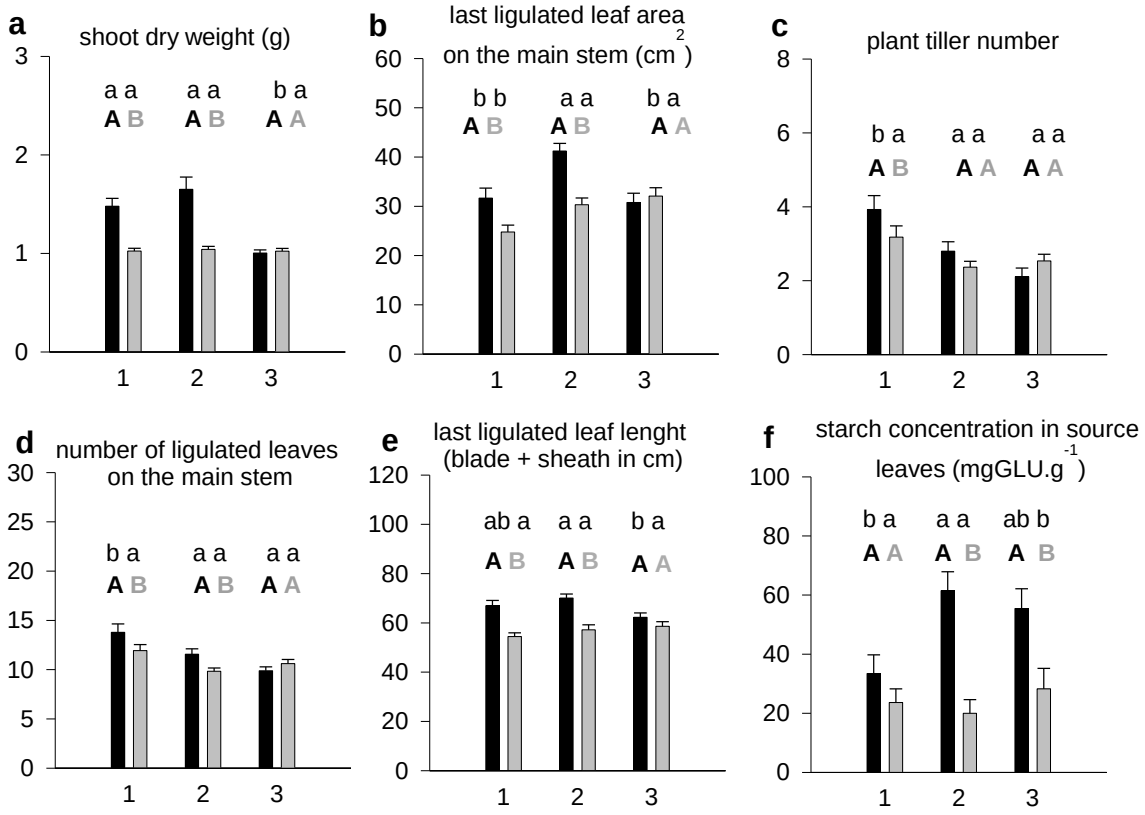

Figure 3 *Bar plots and standard deviations of mean values for each cluster (1, 2 or 3 in abcissa) for morphogenetic variables (not normalized by photo-thermal time or leaf rank) and starch concentration in source leaves (a to f). Black bars represent means for well watered and grey bars for water deficit conditions. Capital letters show the result of a paired t-test for differences between treatments within each cluster at 95% (family-wise confidence level). Different small letters represent the result of a Tuckey multiple comparisons of means among clusters within treatments at 95% confidence level*

DISCUSSION

We studied a wide genetic diversity of rice (43 genotypes) from 5 different sub species, with a majority of tropical-japonica upland rice with different origins (Table 1). This allowed exploring associations among several phenotypic traits that act as component traits for early vigour (here considered as shoot biomass accumulation during exponential growth) and its maintenance under drought. These ensembles of morphogenetic (morphological and phenological) and primary metabolic traits were useful in identifying groups of genotypes having similar characteristics, and possibly adaptation strategies.

Expression and adaptive value of traits related to drought tolerance depend on environment (Tardieu *et al.* 2011). In this study, vegetative plants were subjected to a short dry-down period with a final FTSW' of 0.2. This is a non-lethal stress strong enough to cause a cascade of responses in terms of

stomatal closure, inhibition of expansion and development processes, as well as primary metabolic and enzymatic changes in developing and mature organs (Luquet *et al.* 2008). It represents a short but intense drought spell as it frequently affects upland rice during a time when the plant has not yet gained access to deeper portions of the soil water reserve. We thereby focused on tolerance mechanisms while disabling avoidance (deep rooting or water reserve conservation) from the experimental design, with all plants having extracted the same amount of water at harvest.

All studied component traits showed greater genotypic variance than shoot biomass under stress (Table 2), suggesting that they are potentially useful for breeding. However, since trade-offs among component traits under drought can reduce yield gains (Reynolds *et al.* 2007), the genetic and physiological linkages among them should be understood. Combined consideration of morphogenetic and metabolic traits might help identifying the physiological linkages (Verslues *et al.* 2011).

Components of early vigour in well watered plants are associated with metabolic behaviour

Rebolledo *et al.* (in press) reported a negative constitutive linkage between organ size and number, both contributing to early vigour. In the present sample of genotypes, Cluster 1 derived vigour mainly from organ number (DR and tillering) whereas Cluster 2 derived it from size (Table 7). These types differed in metabolic patterns in source or sink organs. Plants having large leaves and low DR, leaf and tiller number (Cluster 2) showed high concentrations of starch in both source and sink leaves (Table 2 and 7). This group had high sink sucrose and starch concentrations (Table 7) suggesting a small demand for Carbon compared to available assimilates.

Gibson *et al.* (2011) considered starch in leaves as a transient carbon sink enhancing plant growth and photosynthesis. This contrasts with results reported by Sulpice *et al.* (2009) on *Arabidopsis* showing a negative linkage between starch storage and biomass production, similar to our findings Luquet *et al.* (in press) and as observed on Cluster 1. In the absence of physiological stresses and under favorable conditions for Carbon assimilation, low starch concentration in source leaves may thus be a positive trait for vigour in Cluster 1 which has high DR and tillering. An efficient translocation of sugars out of the source toward sink leaves (Vaughn *et al.* 2002) or the storage of an alternative form of carbohydrates reserves instead of starch (He *et al.* 2005) might explain the low amounts of starch in source leaves. However, Cluster 1 did not show significantly higher sucrose levels in source leaves as compared to the other clusters, possibly suggesting that sucrose levels are highly regulated and not used as storage. Rosti *et al.* (2007) observed on a rice mutant deficient in

ADP-glucose pyrophosphorylase (AGPase) in leaf blades abnormally low starch accumulation but normal growth and unchanged sucrose and hexose levels contents in leaf blades.

Rebolledo *et al.* (in press) presented the hypothesis that low-vigour plants may be sink limited, resulting in limited Carbon export from source leaves. In fact low constitutive vigour was generally associated with low SINKSTA despite high concentrations of SOURSTA **(Table 7,** Cluster 3 vs. Cluster 1**)**. The present results suggest that the distinction between source and sink leaves should be important for the interpretation of sugar concentrations. SOURSTA is not necessarily a result of high photosynthetic rates, but sometimes a result of poor translocation to sink organs, as demonstrated for K^+ starved cotton plants (Gerardeaux *et al.* 2011).

Constitutive early vigour is not associated with drought tolerance

Cluster 1 and 2 had greater SDWc accumulation than Cluster 3, which included the smallest plants both in terms of organ size and number under well-watered conditions. However, Cluster 3 was more tolerant to drought in terms of relative reduction of SDW and its components. The constitutive traits providing high early vigour were those showing the greatest relative reduction under drought (eg. tiller number reduction for Cluster 1 in **Table 5; Table 7, Figure 3**). In the present study, large genotypic differences in vigor under well watered were largely leveled under drought (Table 7, Fig. 3). This indicates that vigorous plants were particularly penalized although the final drought intensity was the same for all genotypes. Consequently, the similar final SDW under drought was achieved in Clusters 1 and 2 through constitutive vigour (potential growth) before stress onset and in Cluster 3 by sustaining growth during the drought treatment. This trade-off between potential and tolerance, present even in the absence of physiologically costly avoidance, explains the difficulty to breed to combine tolerance with performance (Kumar *et al.* 2008; Heinemann *et al.* 2011; Serraj *et al.* 2011).

High Carbon reserve status as observed in Cluster 3 may not only constitute a buffer for growth under stress, but also convey dehydration tolerance. Stem reserves were previously reported as an effective yield supporting mechanism under drought for sorghum (Blum *et al.* 1997), wheat (Dreccer *et al.* 2009) and as a tolerance mechanism at the seedling stage in rice (Cabuslay *et al.* 2002) ;

Leaf size and its maintenance under drought is key trait to maintain early vigour

Among the morphological traits, leaf size had the greatest genotypic variance under drought and was significantly reduced compared to well-watered conditions in the entire population **(Table 4)** particularly in genotypes of Clusters 1 and 2 **(Figure 3)**. LDIMc was significantly and positively correlated with biomass maintenance under drought **(Table 5)**. For *Arabidopsis,* Aguirrezabal *et al.* (2006) showed that leaf expansion was more sensitive to drought than leaf appearance rate. By

contrast, in the present study, both traits were sensitive to drought and their degree of sensitivity varied among genotypic groups **(Table 7)**; in addition the maintenance of leaf number and size under drought were positively related whereas the inverse was observed under well-watered conditions **(Table 4)**. This supports results of Tisne *et al. (*2010), showing for *Arabidopsis* the importance of both traits to maintain growth under drought.

Are NSC metabolomics related to growth maintenance under drought?

Sugar related variables contributed significantly to the identification of the three clusters (Table 7), confirming the usefulness of introducing metabolic variables for phenotyping growth behaviour (Meyer *et al.* 2009). The effect of drought on sugar concentrations was also suggested to depend on genotypic differences in carbon acquisition, conversion and utilization (in particular plant growth rate) in *Arabidopsis* (Ramel *et al.* 2009). However, there was no significant, direct correlation between any metabolic trait and SDW under well-watered **(Table 4)** or drought conditions **(Tables 5 and 6)**. Meyer *et al.* (2007) and Muller *et al.* (2011) reported similar findings, supporting the hypothesis that plant growth under drought is essentially sink limited, because structural growth is more sensitive than Carbon assimilation. The alternation between source- and sink-limitation from well watered to drought situation resulted in marked shifts in metabolic pools. It is therefore impossible to predict growth behaviour on the basis of metabolomics alone, as reported by Meyer *et al.* (2007) for *Arabidopsis*. In addition, it is difficult to differentiate between metabolic responses that contribute to stress tolerance from those that are symptoms of stress damage or just represent the accumulation of unused substrates because of reduced growth (Verslues and Juenger 2011).

Nevertheless, growth component traits under drought conditions were associated to sugar related variables **(Table 6)**. Drought increased hexose concentration 2.5-fold for source leaves and 1.3-fold for sink leaves. It reduced SOURSTA by about half, but did not alter SINKSTA. A mantainance of starch in source leaves under drought was associated with a reduction of tiller and leaf number **(Table 6 and Cluster 1)**. Tillering would thus keeps in large part dependent on carbohydrate reserve utilization under drought. Similar results regarding sugar metabolism under drought were reported for drought stressed IR64 rice by (Luquet *et al.* 2008).

The strong increase in hexose levels observed for sink organs under drought was associated with up-regulation of several invertase genes (OsCIN 1, 5, 8 and OsVIN 1, 2 (Luquet *et al.* 2008)) and was thus suggested to be passive, but its physiological function remained unclear. In the present study, changes in hexose concentration induced by stress did not contribute to cluster distinction. In general the measured metabolic variables contributed better to explain constitutive vigour traits (as

observed in well watered plants) than vigour response traits. Nevertheless, they showed significant ($P<0.01$) genotypic differences.

Maintenance of sucrose concentration in source leaves under drought **(Table 7;** Cluster 1 and 3) was previously reported to be related to several drought tolerance indicators: Greater tolerance to atrazine (Ramel *et al.* 2009) and maintenance of phloem loading, a process possibly controlled by an intercellular sucrose signal (Vaughn *et al.* 2002). Recently, it was reported that in Nipponbare rice, with enhanced expression of sucrose transporters (OsSUT2) in source leaves under drought was associated with greater drought tolerance (Ibraheem *et al.* 2011). In our study, sucrose in source leaves was reduced by drought in Cluster 2, consisting of tropical japonicas, but not in Clusters 1 and 3 **(Table 7)**. In fact, tropical japonica upland rices, commonly having large leaves and few tillers, are known for their good drought avoidance but poor physiological tolerance (Lilley *et al.* 1996). According to Farooq *et al.* (2010), however, a trait for large leaves introgressed into a IR64 (indica) background also conveyed physiological tolerance to drought. More research is needed to understand the relationship between organ size sucrose contents and drought tolerance in rice.

CONCLUSION

There is thus evidence that the different response patterns among Clusters, in terms of organogenetic and growt

h vigour, bear a relationship with the regulation of soluble sugar levels, but too little information is currently available for functional interpretation. The practical usefulness of sugar concentrations as metabolic markers of stress adaptation can, at this state of knowledge, not be confirmed. By contrast, under well watered conditions metabolic markers, in particular starch concentration in source leaves are definitely relevant for phenotyping the diversity of rice early vigour. This trait, together with related morphogenetic traits, will be soon included in a genetic association study on a larger (200 accessions) japonica diversity panel including the present materials. The relationships between such metabolic and morphogenetic traits constituting early vigour and yield component traits, in particular fertile tiller number, stay green, and starch remobilization for grain filling, will be also addressed in a forthcoming rice study. These results will benefit to the definition of traits to be accounted for within the GRiSP phenotyping network of the CGIAR.

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Headlines in chapter II

- It was explored within a sub panel of 43 genotypes whether metabolic traits could be used as phenotypic markers of early vigor and its drought regulation.

- Traits conferring early vigor under well watered conditions had a negative impact on drought tolerance (ie. shoot biomass growth maintenance under drought).

- Under drought, the amount of Carbon (C) assimilates in sink and source organs suggested that shoot growth maintenance was not limited in first place by C assimilate availability but rather by sink activity reduction (ie. leaf size,DR and tillering).
- Tillering maintenance however was related to a decrease in starch concentrations under drought.

- Starch and sucrose concentrations under well watered and drought conditions contributed to identify different drought response strategies:
  - Clusters 1 and 2 were advantaged by a constitutive vigour based on DR and leaf size respectively but shared poor drought tolerance; Cluster 3 gathered low vigour genotypes that sustained growth during the drought treatment.

  - Under well watered conditions vigorous genotypes were either source limited, sink driven "bold" plants (Cluster 1: low starch concentrations in source and sink leaves) or sink limited (source driven)"conservative"plants (cluster 2: High starch concentrations in source and sink leaves). Non vigorous genotypes were sink limited (Cluster 3: Low starch concentration in sink leaves and large concentrations of starch in source leaves).

  - Under drought the decrease in starch concentrations in source leaves was lower for "bold" than for "conservative" plants.

- Exploring the physiological traits, related to water use, underlying the negative correlation between early vigour and its maintenance under drought will help understanding how to co-select for both processes. This will be the aim of the next chapter.

Chapter III : Does early vigor occur in combination with drought tolerance and efficient water use in rice genotypes?

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ABSTRACT

Selection for early vigor can improve rice seedlings' access to resources, weed competitiveness and yield. Little is known on trade-offs with drought tolerance. This study explored on a panel of 176 rice genotypes the diversity of traits and trait combinations related to early vigor and water use under drought. In a dry-down experiment (potted plants, 3 replications), plant transpiration and fraction of transpirable soil water (FTSW) were monitored gravimetrically along with plant morphology. The design emphasized tolerance traits while excluding avoidance through deep roots. Plant structural growth was interpolated using Ecomeristem model to calculate leaf level transpiration and its response to FTSW. Broad-sense heritability was high for transpiration rate per unit leaf area, organogenetic developmental rate (DR, syn. phyllochron^{-1}), tiller number and leaf size in well watered, considered constitutive traits. Trait heritability was lower under drought. Specific leaf area (SLA), DR and tiller number were negatively correlated with leaf size. Leaf size, DR and tiller number contributed positively to shoot dry weight and leaf area and thus, vigor. Early vigor was negatively correlated with growth maintenance and WUE under drought, indicating trade-offs. Three clusters of genotypes were identified based on constitutive traits DR, SLA, tiller number and leaf size. Clusters 1 was vigorous and had large leaves, low DR, low tillering, and had high leaf senescence under drought; cluster 3 was vigorous and had small leaves, high DR, high tillering and very sensitive response of transpiration to FTSW; and cluster 2 had poor vigor but superior drought tolerance, associated with small leaves, low DR and low SLA. It had little senescence under drought. In cluster 1, traditional

tropical-japonica upland rices abounded. Cluster 2 had mainly improved genotypes. All lowland and indica rices fell into cluster 3. The naturally occurring trait combinations and their genetic diversity are discussed from a functional and breeding perspective. Further improvement of multi-trait phenotyping approaches is proposed.

INTRODUCTION

During the various phases of rice domestication, cropping systems moved from seasonally flooded habitats to both aerobic and anaerobic rainfed conditions, including drought prone environments (Courtois *et al.* 1999; Liu *et al.* 2004). This resulted in a large agro-ecological and genetic diversity which is of great value today in understanding drought response mechanisms and in improving drought tolerance. Several studies reported constitutive, phenotypic differences between the tropical-japonica group, most commonly grown under aerobic upland conditions; and the indica group, which is more common in aquatic ecosystems (Schlosser *et al.* 2000; Zhao *et al.* 2006c), Tropical japonicas typically having longer and larger leaves, a deeper root system and fewer tillers than Indica rices.

Half of the world's rice production is grown in aerobic upland and rainfed lowland systems, where soil moisture depends on rain and plants are exposed to unpredictable periods of drought (Singh *et al.* 2008). The diversity within and among genetic groups in drought responses was also widely studied (Lilley *et al.* 1996; Schlosser *et al.* 2000; Liu *et al.* 2004; Xu *et al.* 2005; Xu *et al.* 2009). Most authors agree that the sturdy, large-leafed, tropical-japonica upland rice type shows superior adaptation to drought, which has thus become a target ideotype for breeding. But to our knowledge, few studies if any have attempted to use phenomics of diversity panels to identify trait combinations occurring naturally or through breeding, and to relate them to adaptation strategies. Such diversity panels might cover diverse genetic makeup, geographic origin, ecosystem of adaptation (Schlosser *et al.* 2000) and level of improvement (landraces vs. breeding materials and varieties; (Jahn *et al.* 2011)). As opposed to developing ideotype concepts (Xu *et al.* 2009) on theoretical grounds alone, this might help extracting functional concepts from existing diversity, while providing a shortcut to genetics through association studies (Jahn *et al.* 2011; Roy *et al.* 2011).

Early vigor which is a complex trait having morphological, phenological and physiological components lends itself to this approach. The rate of early leaf area development (early vegetative vigor) is determinant for resource colonization and yield competitiveness of the rice seedling (Zhao *et al.* 2006c; Namuco *et al.* 2009; Okami *et al.* 2011)and yield potential (Dingkuhn *et al.* 1999). In a recent study, (Rebolledo *et al.* in press) characterized two rice panels with about 200 accessions for morphological and physiological traits contributing to early vigor. They observed that developmental

rate (DR, defined as the reciprocal of phyllochron), along with tillering and leaf size, is a driver of early vigor under non-limiting water resources. In a related study, Rebolledo et al. (submitted) were able to relate these traits to non-structural carbohydrate (NSC) pools and proposed an empirical typology of genotypes. These groups had different trait combinations contributing to early vigor under well watered conditions and under drought.

For water limited environments, both early vigor and drought resistance are crucial and it is important to find trait combinations that enable both while minimizing trade-offs.Levitt (1986) distinguished between dehydration avoidance and tolerance. Most avoidance traits are morphological and constitutive, thus expressed independently of the stress conditions. Early vigor can contribute positively to avoidance in terms of yield maintenance (Guan *et al.* 2010), but this depends on the phenological timing and severity of drought (Heinemann *et al.* 2011; Sinclair 2011). Early vigor was related positively to yield stability in aerobic systems (Okami *et al.* 2011) but may have no such effect in rainfed lowland environments (Wopereis *et al.* 1996) where avoidance mechanisms such as deep roots cannot always be expressed. In dryland wheat systems, early vigor reduced soil evaporation (Slafer *et al.* 2005) and increased water available for crop transpiration (Rebetzke *et al.* 1999). By contrast, in rainfed lowland situations which are essentially aquatic and therefore require an impenetrable hardpan to store water, drought resistance needs to rely largely on tolerance (Yue *et al.* 2005), which has a different genetic basis from avoidance (Yue *et al.* 2006).
Studies on tolerance traits must experimentally exclude avoidance, such as effects of different root system depth (Cabuslay *et al.* 2002; Kato *et al.* 2008; Parent *et al.* 2010). Setups with restricted soil volume (pots), with pot weight and irrigation amount monitored, enable relating plant observations to current system water deficit in terms of FTSW (Fraction of transpirable soil water) (Sadras *et al.* 1996). With this setup drought tolerance responses for both upland (Luquet *et al.* 2008; Parent *et al.* 2010; Heinemann *et al.* 2011) and lowland rice (Luquet *et al.* 2008; Parent *et al.* 2010) have been reported. The FTSW threshold below which leaf transpiration rate decreases varies among rice genotypes (Luquet *et al.* 2008; Serraj *et al.* 2008; Heinemann *et al.* 2011) and tends to be lower for upland-adapted than for lowland-adapted varieties, indicating a less sensitive stomatal response to FTSW. The orchestration of this and other physiological, phenological and expansion responses to the dry-down process was characterized by Luquet *et al.* (2008). The sensitivity of each such process to FTSW can be considered a tolerance trait potentially involving genotypic differences. How these genotypic differences relate to constitutive phenological and morphological traits within a large spectrum of genetic diversity is little known. We are using phenomics approaches here, in the context of ongoing genetic association studies, to address this research question.

In a previous study (Rebolledo *et al.* Submitted) we related phenotypic variation in early vigor traits (tillering, DR, leaf size) to transient NSC pools under well watered conditions. Constitutively vigorous genotypes with high DR had carbon source limited growth and therefore accumulated less transitory NSC in leaves. Such plants showed strong growth reduction under drought. In the present study we attempt to reproduce these results and relate them to plant traits associated with plant hydrology, such as sensitivity of stomatal response to FTSW.

Using a panel of 176 rice genotypes mainly composed of tropical japonica types, this study investigates to which extent constitutive early vigor is associated with drought tolerance during vegetative growth, and whether vigor-tolerance trade-offs observed within the genotypic spectrum can be explained with water-use related traits. Results are discussed in view of identification of drought adaptation strategies for crop improvement

MATERIALS AND METHODS

Plant material

The experiment was conducted in a greenhouse of IRRI, Los Banos, Philippines (14° 11'N, 121°15'E) during the wet season (September-November) of 2010. Seeds of 176 rice (*Oryza Sativa* L.) accessions were germinated in Petri-dishes in a germination chamber at 29°C. When the coleoptiles attained 2 cm, five seedlings were transplanted to 1-L plastic pots containing 930 g (dry weight) of Maahas clay soil (Isohyperthermic Typic Hapludalf) with available P (Olsen) 43.2 mg.Kg^{-1} , 36.2% CLay, 22.5% Sand, 41.5% Silt, exchangeable K 1.14 in mEquiv/100g , 0.17% total N (Kjeldahl) and a PH of 5.8 (H_2O). Relative soil humidity (wet-dry)/dry) at wilting point was 0.15 and at field capacity 0.62. Complete fertilizer (14N,14P,14K) at a rate of 145mg/pot and additional ammonium sulfate (21-0-0) at a rate of 50mg/pot was applied as a single application before transplanting, and Ferrous sulfate solution (3%) and Manganese sulfate solution (4%) were applied as foliar spray two weeks after transplanting.

Pots were arranged in a block design, each block corresponding to one replicate. Within each block, genotypes were randomly distributed in pairs of pots, the two pots receiving different treatment (well watered and water stressed). In each pot plants were thinned to two seedlings after one week of culture, and then to one seedling at four leaf stage. The remaining plant was used for measurements. The mean distance between plants was 20 cm. Three replicates (pots) were used per cultivar and treatment. Weather data were acquired with one station in each block. In each station air temperature (T) and relative air humidity (RH) were continuously recorded with hourly integration at the top of the plants using HMP 45C (Vaisala, Finland) sensors shielded from direct light and exposed to free air circulation. Photosynthetically Active Radiation (PAR) was hourly measured using quantum sensor SPK 215 measured at the top of the plants and registered at hourly integration.

Mean minimum and maximum T were 25°C and 36°C, respectively, mean minimum RH was 65% and calculated mean daily incident global radiation calculated from PAR was 6.97 $MJ.M^{-2}$. The T was used to compute thermal time (TT) as the sum of daily mean temperature (hourly integration) minus a base temperature of 12°C (Gao *et al.* 1992).

176 accessions obtained from IRRI's germplasm bank (80%), and the rest obtained from Sénégal, Mali and CIRAD were multiplied in a greenhouse in CIRAD and then used for this experiment. The panel included a genetically wide spectrum of 152 tropical japonica rices and a nucleus of 17 genotypes including 5 tropical Japonica, 5 indica, 3 temperate japonica, 2 aus and 2 aromatic based on the izonenzyme classification described by (Glaszmann *et al.* 1984). Genotypes represented improved varieties or breeding lines (n=73) or traditional (n=101) cultivars and were associated with three different hydrological ecosystems of adaptation: Irrigated lowland (n=12), rainfed upland (n= 149) and rainfed lowland (n=6), lowland environments being partly or permanently flooded and upland environments being drained and aerobic (Table 8).

Plant measurements

Plants were characterized by the number of leaves appeared on the main stem (LN_MS), plant tiller number (NBT), number of ligulated leaves in the main stem (NBL), the leaf area of the last ligulated leaf in the main stem (Leaf length of blade (LL), blade width (LW) multiplied by an empirical allometric coefficient 0.725) and the length of the expanding leaf (Lexp). LN_MS, LL and Lexp were used to compute Haun Index (HI) (Haun 1973), phyllochron and its reciprocal Developmental Rate (DR), (following Eq.1) between two dates (Δ).

$$DR = \Delta HI / \Delta TT \ [°C.d^{-1}] \qquad \text{(Eq.1)}$$

DR was computed for the period from germination until onset of drought treatment at the 6-leaf stage (DRinit) and from treatment onset to the end of the treatment (DRonset) for stressed and well watered plants.

At the end of the experiment and for each treatment, plants were sampled to measure shoot dry weight . Dry weights for bulk plant green leaf blades and the last ligulated leaf blade were measured separately. Plant green leaf blade area was measured with a planimeter (LI-COR 3100). Specific leaf area (SLA in $cm^2.mg^{-1}$) of the same leaf was estimated dividing computed leaf area of the last ligulated leaf by its measured dry weight.

Observations of SDW with a destructive sampling were conducted on control and stressed plants at the end of the drought cycle. Plants obtained by thinning at 4^{th} leaf stage for each replicate and genotype were measured to obtain SDW1 at the onset of drought treatment. We used a multiple

linear regression model across all genotypes and replicates (Eq.2) to estimate SDW1 based on a highly significant ($p<0.001$, $R^2=0.85$) correlation between SDW and LNB, LL, Lexp and SDW (Eq.2)

$$SDW1 = -0.84 + 0.014\,\mathrm{LL} + 0.005\,\mathrm{Lexp} + 0.061\,\mathrm{LNB} \qquad (\mathrm{Eq}\ 2.)$$

Leaf senescence on the plant (SEN) was estimated as the proportion of dead leaf number (i.e. leaf with dead tissue > 50% of the leaf surface) relative to total leaf number in the plant. Leaf rolling (ROLd) was estimated in water stress treated plants following an established scoring system ranging from 1 to 9 (IRRI 1996) for all green leaves at the end of the drought treatment.

Variables measured on control well watered plant were indexed as 'c' (eg., SDWc) and those measured on water deficit plants as 'd'. The response to drought calculated for each variable (dc/c) was computed as the difference between'd' and 'c' values divided by the 'c' value (Eq 3).

(Eq. 3):

$$\mathrm{VAR}d_{C/C} = \frac{(d - c)}{(c)}$$

Normalization of phenological and morphogenetic variables

The date of drought treatment onset was chosen by phenological stage (6th leaf) and the treatment was ended when a certain soil water deficit was attained (FTSW' = 0.2). In order to compare plants having different age at stress onset and final sampling, growth variables tiller number(NBT), green blade area (PLA) and shoot dry weight (SDW) were normalized by the photothermal time (PT) (Eq. 4) accumulated from germination to these dates (see (Rebolledo *et al.* in press) for details).

$$PT = \left(\sum_n PAR\right) \times TT \text{ , in MJ.°C.d.m}^{-2} \qquad (\mathrm{Eq.4})$$

Leaf area of the last ligulated leaf was normalized by its rank and named LDIM (see (Rebolledo *et al.* in press) for details). Using a model determined for cv. Nipponbare, the variation of SLA as a function of leaf rank on the main stem was determined, supposing that SLA exhibits non-linear descending pattern with leaf position (Luquet *et al.* 2007). For comparison among genotypes, SLA was thus normalized for the 7th leaf on the main stem.

Drought treatment

The dry-down system was described in detail by Luquet *et al* (2008) and Rebolledo *et al.* (Submitted). The drought treatment was initiated at the 6th leaf stage for each genotype individually. At that time, pots of drought treated plants were watered to field capacity, then sealed with plastic film to avoid evaporation from soil surface and drainage holes, and from then on weighted once a day before

10AM to monitor water loss. The rate of water loss was used to estimate plant transpiration rate and the accumulated loss was used to calculate FTSW'. By contrast with FTSW of Luquet *et al* (2008) , FTSW' was calculated since it considers the same Wilting Point value for all genotypes. The FTSW' was calculated following Eq. 5:

$$FTSW' = \frac{AW - WP}{FC - WP} \qquad \text{(Eq.5)}$$

In Eq.5, AW is the actual weight of a given pot whereas WP and FC respectively correspond to pot weight at wilting point and field capacity, which were known on the basis of the quantity of soil used and the weight of pots and plastic film. Plant measurements were performed at FTSW' 1 (stress onset) and 0.2 (targeted stress level for final sampling) for stressed plants and their respective well watered check.

Water Use Efficiency under drought

For water stressed plants, water use efficiency (WUEd in $g.kg^{-1}$) was calculated for each replicate as the difference in shoot mass divided by the total water transpired during the dry down period. To calculate WUEd we used the estimated SDW1 (Eq.2) at the beginning of the treatment, the measured SDWd (non normalized by PT) at the end of the treatment (ie. At FTSW' 0.2) and the weight of the soil at FTSW' 1 (AW 1) and 0.2 (AW 0.2) (Eq. 6):

$$WUE = \frac{SDWd - SDW1}{AW0.2 - AW1}$$

Critical FTSW' for decrease in leaf transpiration

During dry down, daily transpiration (TR) was calculated as the difference in weight between successive days. Varieties reach at different days the 6th leaf stage (Stress onset date), then although the dry down duration from FTSW' 1.0 at 6th leaf stage to FTSW' 0.2 was between 11 to 16 days, the study of the entire panel last 35 days. In order to take into account the effect on TR of potential evapotranspiration (PET), which is its driving force, daily normalized TR was calculated as TR divided by PET. PET was calculated at a daily time step with the Penman-Monteith equation accordingly to the method of Allen *et al.* (1998). Mean values for PET were 1.72$mm.day^{-1}$ (Sd 0.4).

In order to express TR on a per-leaf area basis because plants varied in size, a second normalization was performed by dividing TR by PLA. The model Ecomeristem (Luquet *et al.* 2006), simulating water-limited plant growth at organ level (Luquet *et al.* 2008a) was used to interpolate daily values of PLA from stress onset to the end of stress day for each plant (replicate) individually. For this purpose,

Ecomeristem was fitted by parameter optimization on no normalized available data (NBT, NBL, LDIM, SEN, SDW, PLA at FTSW 1.0 and FTSW 0.2). Cases with simulation error >12% across replicates were discarded. Consequently only 88 genotypes were retained for analysis of response of TR (normalized by interpolated PLA) to FTSW.

Across genotypes and replicates there was no significant effect of FTSW on TR for FTSW > 0.8, TR being considered as being maximal under these conditions. The maximal transpiration rate TRmax was thus calculated as the average of TR observations at FTSW > 0.8 for each individual plant. TRmax was considered a constitutive genotypic trait in this study, and it was also used to express TR on day(i) as a fraction of TRmax (normalized transpiration rate, NTR).

The critical FTSW' for onset of decrease of transpiration (FTSWthres) was calculated as follows. NTR was plotted against FTSW' for each genotype using pooled daily data from 3 plants (replicates), in order to dispose of sufficient points to analyze the response. A composite, bilinear regression analysis consisting of a plateau and a sloping branch was implemented using R software (http://www.R-project.org), with FTSWthres defined as the point where the two lines meet. The slope parameter of the correlation (Slope) thereby indicates the rate at which TR decrease per unit of decrease in FTSW'. The regression thus fitted two linear segments where one segment is a plateau *y*=1 and the second is a linear change in *y* with respect to *x* (Eq.7)

$$If\ FTSW\ \geq\ FTSWthres\quad y\ =\ 1$$

$$Else\ y\ =\ Slope\ (x\ -\ FTSWthres) + 1 \qquad \text{Eq. 7}$$

Statistical analysis

Analysis of variance was carried out using the GLM (General linear model) procedure in SAS for windows V.9.2 (Statistical Analysis System institute, Cary, NY, USA), to determine differences between treatments control (c) and drought (d). Broad-sense heritability H^2 (Gallais 1990) was computed from the estimates of F-value based on one-way analysis of variance (replicate; genotype) using *aov* function in R (http://www.R-project.org), following Eq 8.

$$H2\ =\ \theta\text{G2}\ *\ (\theta\text{G2} + \theta\text{E2}/n) \qquad \text{Eq. 8}$$

Where θ_G^2 and θ_E^2 are estimates of genetic and residual variances respectively and *n* is the number of replicates.

Spearman correlation analysis among variables and across genotypes was performed using R. A cluster analysis was also conducted to identify groups of rice genotypes having similar value combinations for constitutive (well watered treatment based) variables contributing to early vigor (DRinit, LDIMc, NBTc, SLAc). Clustering was performed using all the constitutive variables with FactoMiner package in R following (Husson *et al.* 2011), refer to (Rebolledo *et al.* Submitted) for details. Differences among clusters were studied using the student T-test, and Chi-test was used to test the relationship among categorical variables (e.g., frequency of breeding history or ecosystem adaptation categories among clusters).

Goodness of fit of the bilinear plateau regressions was estimated by calculating the coefficient Modelling Efficiency (ME) as described by (Wallach *et al.* 2006) (Eq 9) where n is the number of observations, Yobs are the observed points, Ypred are the points predicted by the plateau regression and Yobsemean is the mean for the observations.

$$ME = 1 - \frac{\sum_{i=1}^{n}(\mathrm{Yobs} - \mathrm{Ypred})^2}{\sum_{i=1}^{n}(Y\mathrm{obs} - Y\mathrm{obsmean})^2} \qquad \mathrm{Eq.}\,9$$

A p-value indicating the significance of each parameter (FTSWthres, Slope of the regression) was computed. For each regression analysis a standard error (SE) was associated with the estimated parameters (FTSWthres, *Slope*). Only regressions with ME >0.5, FTSWthres and Slope of regression with p-values <0.01 were considered.

RESULTS

Drought differentially affects vigor traits but heritability of constitutive traits is greater

Plant variables measured at FTSW' 0.2 were significantly reduced ($p<0.01$) as compared to controls: SDW, PLA, LDIM, DRonset, NBT (Table 1). Drought increased SLA, thus causing a weight loss per unit area. On average, drought resulted in a 52% reduction in plant leaf area (PLA dc/c) and a 45% reduction in dry weight (SDWdc/c) (Table 2). The variables most strongly affected by water deficit were LDIM and SEN, followed by DRonset, NBT, and SLA (Table 2). There was no difference between stressed and control plants for variables measured before stress onset, such as DRinit (Table1).

All constitutive morphological traits (i.e. measured under well watered conditions) had higher heritability than traits measured under stress (Table 1) and response traits (Table 2). Constitutive traits (except SDWc, PLAc and DRonsetc) had broad-sense heritability >0.5, and for LDIMc, NBTc, DRinitS heritability was >0.7.

A large variation across the 176 accessions was observed for DRinit that ranged from 0.013 to 0.0205 for well watered plants (DRinitc) and from 0.0138 to 0.204 °Cd-1 for stressed plants (DRinitd) (Table 1). TRmax, expressed as fraction of potential evapotranspiration (PET), ranged from 1.2 to 5.6 (Table 2, Fig. 1).

Under stress NBTd and LDIMd showed the highest heritabilities, even higher than that observed on other variables measured on well watered plants (Table 1). There was a twofold difference in tiller number between the genotype with the highest and smallest tiller number, and a sevenfold difference for LDIMd (Table 1). Large genotypic variation was therefore observed for LDIM response to drought, with a heritability of LDIM dc/c of 0.45 (Table 2), indicating that genotypes differed strongly in leaf size reduction under drought.

Among the water-use related variables, heritability of WUEd was 0.4 and that of TRmax 0.79, associated with a large range of genotypic variation (Table 2, Fig.1).

Table 1. Broad sense heritability (H^2), mean, standard deviation (Sd), minimum and maximum values for variables measured under well watered (c) and drought stressed plants (d) on 176 genotypes. For each variable (mean for 3 replicates), letters indicate the significance of mean comparison (Tuckey test; p<0.01) between treatments well watered (c) and water deficit (d).

Variable	H^2	Mean		Sd	Minimum	Maximum
SLAc	0,55	325,96	a	45,36	218,47	596,42
SLAd	0,53	289,55	b	48,18	177,94	444,35
LDIMc	0,83	6,75	a	1,42	2,83	10,06
LDIMd	0,74	1,29	b	1,29	1,21	7,86
DRinic	0,88	1,73E-02	a	1,35E-03	1,30E-02	2,05E-02
DRinitd	0,87	1,74E-02	a	1,25E-03	1,38E-02	2,04E-02
DRonsetc	0,39	1,61E-02	a	2,80E-03	8,72E-03	2,34E-02
DRonsetd	0,23	1,10E-02	b	2,87E-03	2,73E-03	1,86E-02
NBTc	0,85	6,95E-05	a	2,26E-05	2,96E-05	1,68E-04
NBTd	0,77	4,76E-05	b	1,41E-05	2,55E-05	1,08E-04
SDWc	0,52	2,12E-05	a	5,16E-06	9,93E-06	3,80E-05
SDWd	0,68	1,06E-05	b	2,08E-06	4,79E-06	1,78E-05
SENc	0,33	0,07	a	0,03	0,00	0,17
SENd	0,32	0,13	b	0,10	0,00	0,52
PLAc	0,68	3,33E-03	a	8,48E-04	1,07E-03	6,19E-03
PLAd	0,38	1,43E-03	b	4,22E-04	4,24E-04	2,77E-03

Table 2 Broad sense heritability (H^2), mean for three replicates, standard deviation (Sd), minimum and maximum values for drought response variables (indexed as dc/c), rolling index (ROLd) and Water Use Efficiency (WUEd: $g.kg^{-1}$) under drought and plant transpiration rate at stress onset (FTSW of 1; TRmax as a fraction of evapotranspiration) measured on 176 genotypes.

Variable	H2	Mean	Sd	Minimum	Maximum
WUEd	0,40	1,72	0,47	0,49	3,21
TRmax	0,79	0,279	0,082	0,12	0,56
ROLd	0,33	3,01	2,18	0	9
$SDW_{dc/c}$	0,31	-0,45	0,16	-0,86	0,39
$NBT_{dc/c}$	0,19	-0,27	0,17	-0,62	0,33
$DRonset_{dc/c}$	0,05	-0,29	0,2	-0,81	0,41
$LDIM_{dc/c}$	0,45	-0,32	0,18	-0,74	0,71
$SLA_{dc/c}$	0,19	-0,09	0,16	-0,61	0,38
$SEN_{dc/c}$	0,29	0,9	1,29	-1	7,82
$PLA_{dc/c}$	0,09	-0,52	0,21	-0,9	0,39

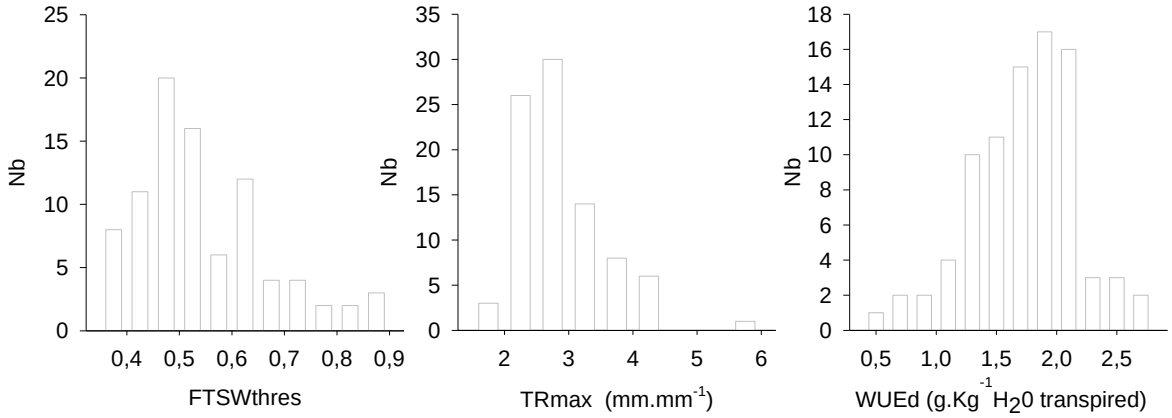

Figure 1. *Distribution in a subset of 88 genotypes (number of individuals: Nb) for mean values of Water Use Efficiency under drought (WUEd), plant Transpiration Rate at stress onset (TRmax as a fraction of potential evaporation rate PET) and FTSW threshold below which plant transpiration rate start decreasing (FTSWthres) estimated with the plateau regression in equation 7.*

Correlations between traits observed for control and drought plants

For variables measured on controls, all morphological traits measured were significantly ($p<0.01$) correlated with PLAc (Table 3). The same trend was observed for SDWc, but surprisingly SLAc was not correlated with SDWc (Table3). SLAc was positively and significantly ($p<0.01$) correlated with organ number related variables (NBTc, DRinitd) but was not related to organ size (LDIMc), nor to SENc (Table 3).

Similar to a previous, independent study (Rebolledo *et al.* in press), the organogenetic developmental rate (DRinitd) and tiller number (NBTc) were positively correlated with SDWc and PLAc, indicating that early vigor was driven by leaf and tiller production rate. The negative correlation between leaf size (LDIMc) and tiller number was also confirmed.

A partial spearman correlation matrix (Table 4) was used to explore the relationship between traits related to plant constitutive early vigor, water use and their response to drought. While transpiration rate at stress onset (TRmax) was not significantly correlated with any morphological variable (Table3), it was significantly positively correlated with FTSWthres and negatively correlated to the slope of the regression. Consequently, genotypes having high maximal transpiration rate reduced transpiration early on during stress. No correlation, however, was found between TRmax and the WUEd observed during the drought cycle (Table 4). The negative correlation between FTSWthres and the slope of the descending branch of the broken-stick TR vs. FTSW regression (ie.slope of the regression) is inherent to this type of analysis and thus has no physiological significance (Table 4).

All drought response variables were significantly and negatively correlated with their corresponding variables measured under well-watered conditions (Table 4). Constitutive variables showing a non significant correlation with SDWdc/c were SLAc and LDIMc. However, LDIMc showed a significant ($p<0.05$) and negative correlation with PLAdc/c, FTSWthres and a positive correlation with NBTdc/c. On the opposite DRinitd was significantly and positively correlated to FTSWthres.

WUEd was significantly and negatively correlated with constitutive morphological traits such as SLAc, PLAc, NBTc and DRinitd (Table 4). WUEd was positively and significantly correlated with SDWdc/c (0.21, pvalue<0.05), indicating that superior maintenance of biomass was associated with greater water use efficiency under drought. But no correlation was found between WUEd and parameters of transpiration response to drought or to the maintenance of morphological traits under drought (data not presented).

Table 3. Spearman correlation matrix for average variables measured under well watered conditions (c). Mean variables on 3 replicates and 176 genotypes. Bold values indicate significant correlations (p<0.05).

	SDWc	PLAc	SLAc	LDIMc	NBTc	DRinitd	TRmax	SENc
SDWc	**1,00**	**0,81**	0,02	**0,23**	**0,35**	**0,34**	0,00	**0,32**
PLAc	**0,81**	**1,00**	**0,21**	**0,26**	**0,43**	**0,40**	0,00	**0,17**
SLAc	0,02	**0,21**	**1,00**	-0,09	**0,31**	**0,30**	-0,04	0,00
LDIMc	**0,23**	**0,26**	-0,09	**1,00**	**-0,47**	**-0,30**	-0,14	**0,31**
NBTc	**0,35**	**0,43**	**0,31**	**-0,47**	**1,00**	**0,44**	-0,02	**-0,26**
Drinitd	**0,34**	**0,40**	**0,30**	**-0,30**	**0,44**	**1,00**	0,09	-0,02
TRmax	0,00	0,00	-0,04	-0,14	-0,02	0,09	**1,00**	-0,13
SENc	**0,32**	**0,17**	0,00	**0,31**	**-0,26**	-0,02	-0 ,13	**1,00**

Table 4. Partial spearman correlation matrix between average variables measured under well watered conditions (c), drought response variables (indexed as dc/c) and variables related to plant water use response to drought (WUEd and variables estimated in equation 7). Mean variables on 3 replicates and 176 genotypes. Only correlations involving FTSWthres, TRmax or Slope are based on 88 genotypes. Bold values indicate significant correlations (p<0.05).

	SDWc	PLAc	SLAc	LDIMc	NBTc	DRinitd	TRmax
$SDW_{dc/c}$	**-0,72**	**-0,50**	-0,07	0,01	**-0,35**	**-0,31**	-0,09
$PLA_{dc/c}$	**-0,56**	**-0,52**	-0,15	**-0,21**	-0,15	**-0,30**	-0,05
$SLA_{dc/c}$	0,12	0,11	**-0,26**	-0,15	0,16	**0,18**	0,00
$LDIM_{dc/c}$	**-0,39**	**-0,31**	**-0,17**	**-0,23**	-0,06	**-0,30**	**-0,17**
$NBT_{dc/c}$	**-0,20**	**-0,17**	-0,04	**0,20**	**-0,53**	**-0,18**	0,03
$Dronset_{dc/c}$	**-0,31**	**-0,24**	-0,16	0,03	-0,09	**-0,18**	0,14
WUEd	-0,14	**-0,18**	**-0,39**	0,07	**-0,39**	**-0,18**	-0,03
Slope	-0,11	-0,12	-0,03	**0,26**	-0,08	**-0,32**	**-0,38**
FTSWthres	0,09	0,01	0,01	**-0,23**	0,09	**0,28**	**0,52**
$SEN_{dc/c}$	0,09	0,04	**0,18**	0,09	-0,04	0,11	0,12

Do genotype groups having common constitutive traits have common drought responses?

Component traits of constitutive early vigor were used to divide the 176 genotypes studied into clusters having similar behavior. Three clusters were defined using variation in SLAc, NBTc, LDIMc and DRinitd, which are principal morphological and phenological traits constituting early vigor of the shoot (Table 3). Cluster 1 was distinguished by large leaves, low tiller number and slow organogenetic development (DR). Cluster 3 had opposite characteristics: small leaves and rapid development. Cluster 2 was intermediate in terms of tillering and DR, but had small and comparatively thick leaves (low SLA) (Table 5, **Figure 3**).

These clusters differed ($P < 0.05$) also in many traits that did not participate in cluster definition. Judging from the more integrative variables describing biomass (SDWc) and leaf area (PLAc), the high-tillering cluster 3 was the most vigorous under control conditions, and cluster 2 was the least vigorous ($P<0.05$; Table 5). But cluster 2 stood out by exhibiting the greatest drought tolerance, as indicated by the smallest relative change under drought in SLA (SLAdc/c), leaf size (LDIMdc/c), plant leaf area (PLAdc/c) and leaf senescence (SENdc/c). Cluster 3, the most vigorous, was the least drought tolerant, particularly in terms of biomass reduction (SDWdc/c) and leaf area reduction (PLAdc/c), although the big-leafed cluster 1 had the greatest leaf senescence under drought (SENdc/c; Table 5).

No differences among clusters were found for the maximal transpiration rate (TRmax), however the vigorous but drought intolerant cluster 3 had a particularly sensitive response of transpiration to drought, as indicated by a high FTSW threshold for transpiration (FTSWthres) (Table 5, **Figure 2**). Cluster 3 also had the lowest ($P<0.05$) water use efficiency during the drought period (WUEd; Table5).

Only 88 out of the 176 accessions could be evaluated for FTSWthres using a broken-stick bilinear regression model applied to daily data pooled from 3 replications describing the transpiration rate vs. FTSW relationship. For the remaining accessions, the number of observations was insufficient to establish a significant correlation at $P<0.05$. Contrasting examples of transpiration rate vs. FTSW scatterplots are presented in Figure 2, namely, three cases each for low (cluster 1), intermediate (cluster 2) and high FTSWthres (cluster 3). The examples represent extreme cases and thus dare not representative of the mean behavior of genotypes of the clusters.

The three examples from large-leafed cluster 1 are tropical japonica upland rices from Brazil (Guarani), Madagascar (Komojamanitra) and Nigeria (OS4), known for their drought avoidance capacity through deep roots. The three examples from drought tolerant cluster 2 are improved tropical japonica rices from various locations in South America, whereas the three examples for high-

tillering cluster 3 are improved indica materials from the International Rice Research Institute (IRRI) in the Philippines: IR64 is a high-yielding variety grown world-wide in irrigated systems, IR72967-12-2-3 is one of the highest yielding New Plant Type (NPT) lines of IRRI, and 62667 (syn. IR 51089) is a rainfed-lowland adapted line of IRRI. The examples from cluster 3 have in common a reduction of transpiration rate already at very mild levels of drought (**Figure 2 f-h-i**), the New Plant Type line having no plateau at all, indicating an extremely sensitive response of stomata to soil water deficit. By contrast, the examples given for cluster 1 have a large plateau and transpiration rates decrease when FTSW drops well below 0.5. Most accessions of the panel showed intermediate behavior, but the results indicate that substantial genetic diversity exists in rice in terms of regulation of transpiration rate under water deficit. This diversity also includes cases of a negative slope on the plateau as seen in **Figure 2** for cv. Primavera, indicating that TRmax is not attained at full soil water saturation but at mild soil water deficit, as sometimes observed in water-logging susceptible genotypes. These rare observations, which resemble results reported by Luquet *et al.* (2008), were not considered in the present analysis.

Figure 3 presents an overview of mean cluster characteristics under control and drought conditions. The superior drought tolerance of cluster 2 did not result in superior biomass and leaf area at the end of the drought treatment because it had inferior constitutive vigor under control conditions. In fact, differences observed in SDW, PLA and DR under control conditions disappeared during drought (**Figure3**). Differences among clusters in tiller number, leaf size and SLA ($P<0.05$) were maintained under drought but they became smaller in absolute terms. It is noteworthy that cluster 2 had significantly less senescence ($P<0.05$) than cluster 1 under both control and drought conditions.

Cluster composition is related to adaptation to ecosystem and breeding history

Table 6 presents the distribution of genotypes among clusters according to their ecosystem of adaptation and breeding history (improved vs. traditional). The association of these categories with clusters, as opposed to random distribution of categories within clusters, was significant (Chi test: $p<0.01$), in a decreasing order of significance: ecosystem of adaptation, breeding history and finally genetic group. The large-leafed cluster 1 consisted exclusively of upland-adapted genotypes, but many upland-adapted genotypes were also found in the drought-tolerant, small-leafed cluster 2. Upland-adapted genotypes were least frequent in the high-tillering, vigorous, drought intolerant cluster 3.

Because the population studied here was composed to study the phenotypic diversity of tropical japonica rices, only 12 irrigated-lowland adapted accessions were included. These fell predominantly into cluster 3 and none were found in cluster 1. Only 6 rainfed-lowland adapted accessions were

investigated, but none of them were associated with cluster 1. Consequently, none of the 18 accessions adapted to aquatic conditions (irrigated or rainfed lowland) occurred in cluster 1.

Regarding breeding history, improved materials occurred predominantly in cluster 2, followed by cluster 3. Traditional varieties, on the other hand, were most frequent in cluster 1 and rare in cluster 2. Cluster 3 was about equally populated with improved and traditional materials.

The population investigated consisted of a large panel covering the genetic diversity of the tropical japonica group and a nucleus of 17 genotypes (Perlegen panel, corresponding to the OryzaSNP panel (McNally *et al.* 2009)° covering all genetic groups of *Oryza sativa* L. On this basis, no statistical analysis was possible to test for the association of genetic groups, with the 3 clusters. It is noteworthy, however, that both aromatic-type rices and 4 out of the 5 indica rices on this sub-panel belonged to cluster 3, whereas the 2 AUS rices did not (Table 7).

Table 5: Summary of the cluster analysis with mean values and standard deviations (Sd) for each cluster for variables measured under control conditions and drought response variables (indexed as dc/c). Multiple comparisons of means among groups at 95% confidence level (Tuckey test) are presented by different small letters.

Variables	Cluster 1		Cluster 2		Cluster 3	
	Mean	Sd	Mean	Sd	Mean	Sd
Constitutive variables used for cluster definition						
SLAc	325,07	47,34 **c**	299,85	33,39 **a**	354,37	36,23 **b**
LDIMc	8,05	0,81 **b**	5,77	0,85 **a**	5,92	1,12 **a**
NBTc	5,50E-05	1,32E-05 **c**	6,64E-05	1,42E-05 **a**	9,31E-05	2,10E-05 **b**
DRinitS	1,67E-02	1,04E-03 **c**	1,73E-02	8,05E-04 **a**	1,86E-02	9,72E-04 **b**
Constitutive variables measure of early vigor						
SDWc	2,09E-05	4,84E-06 **a**	1,89E-05	4,42E-06 **a**	2,40E-05	5,07E-06 **b**
PLAc	3,34E-03	7,90E-04 **c**	2,85E-03	7,11E-04 **a**	3,82E-03	7,86E-04 **b**
Drought Response variables						
SDWdc/c	-0,44	0,13 **a**	-0,4	0,17 **a**	-0,54	0,13 **b**
PLAdc/c	-0,54	0,17 **b**	-0,42	0,24 **a**	-0,6	0,18 **b**
SLAdc/c	-0,12	0,15 **b**	-0,05	0,15 **a**	-0,09	0,18 **ab**
LDIMdc/c	-0,33	0,15 **b**	-0,24	0,19 **a**	-0,38	0,19 **b**
NBTdc/c	-0,23	0,17 **a**	-0,27	0,18 **ab**	-0,34	0,14 **b**
SENdc/c	1,14	1,49 **b**	0,53	0,92 **a**	0,92	1,24 **ab**
Water-use related variables						
WUEd	1,81	0,48 **a**	1,86	0,32 **a**	1,46	0,50 **b**
FTSWthres	0,52	0,10 **a**	0,54	0,11 **ab**	0,60	0,15 **b**
TRmax	2,65	0,73 **a**	2,90	0,83 **a**	2,85	0,92 **a**

Table 6 Partial contingency Table. Categories and modalities for breeding type (I: Improved, T: traditional), Ecosystem of adaptation (I: Irrigated, U:Upland, RL: Rainfed lowland) within clusters (described in Table 6), considering the 176 genotypes of the studied panel .NA is the number of genotypes with unknown classification for each category. For subspecies (Aro: Aromatic, Aus, Indica, TempJap: Temperate japonica, TropJap: Tropical Japonica) the table was build based on the 17 genotypes constituting the Perlegen reference nucleus (McNally et al. 2009). The number of individuals from each cluster within each category is indicated. Relative frequency by cluster for each category is indicated.Bold values indicate modalities for which Chi- test was significant, suggesting as link between modalities from constitutive cluster and categories.

Category		Rel. frequency by cluster (% of category sample)			Nb. accessions per category
		1	**2**	**3**	
Breeding history (n=176)	**I**	22	**48**	30	73
	T	**54**	**18**	28	101
	NA				2
Ecosystem (n=176)	**I**	0	17	**83**	12
	U	**47**	32	21	149
	RL	0	33	67	6
	NA				9
Genetic group [Perlegen diversity sample] (n=17)	**Aromatic**	0	0	100	2
	Aus	50	50	0	2
	Indica	0	20	80	5
	Temp. Jap.	0	33	67	3
	Trop. Jap.	60	20	20	5

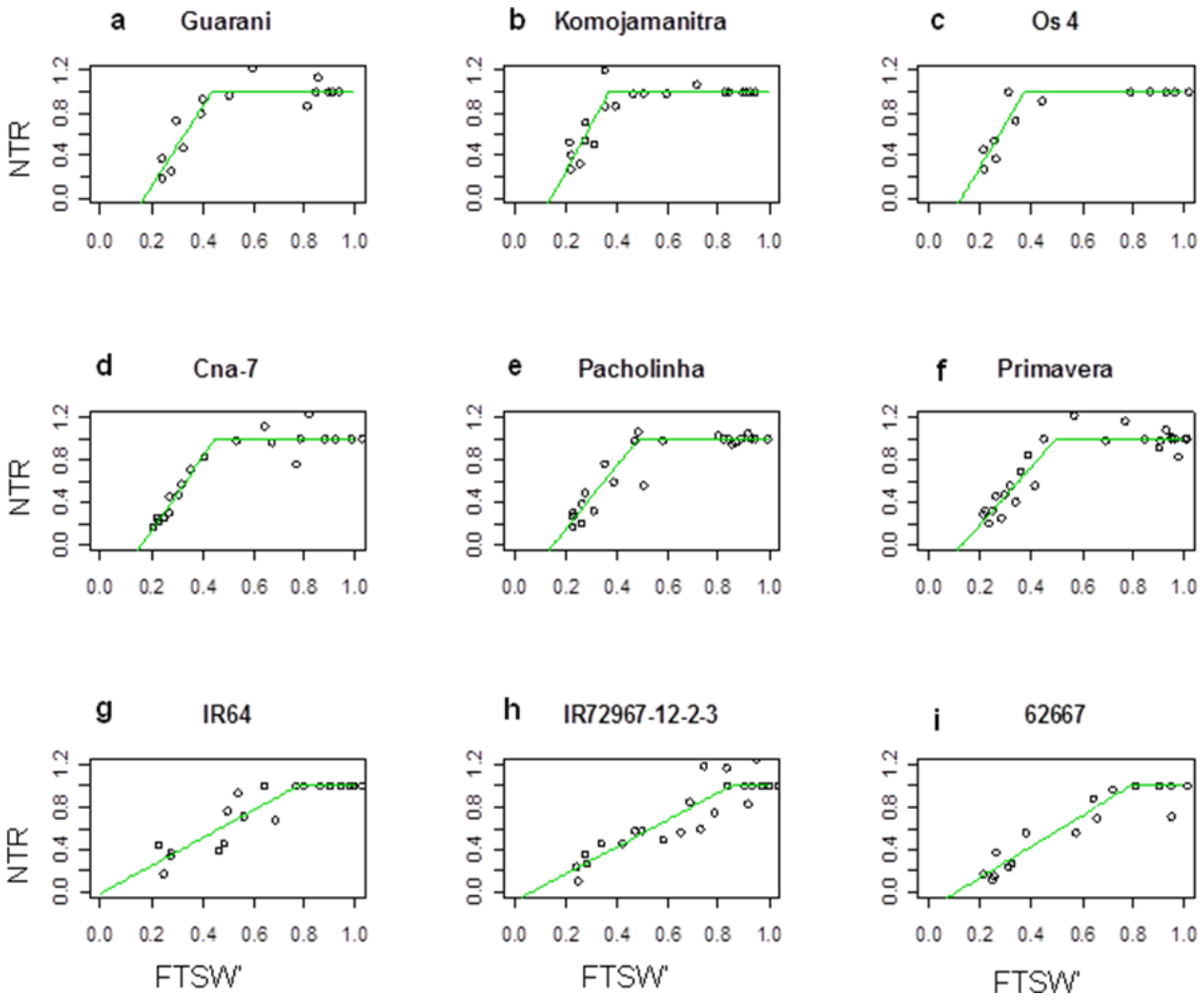

Figure 2 *Relationship between daily plant transpiration rate expressed as a fraction of potential evapotranspiration rate (PET) normalized by TRmax, with fraction of transpirable soil water (FTSW'); for 3 genotypes each having low (abc), intermediate (def) and high (ghi) critical FTSW triggering decrease of transpiration rate. Genotypes abc belong to cluster 1, def to cluster 2 and ghi to cluster 3 according to classification by traits constituting early vigor. Solid lines indicate bilinear correlation using a broken-stick model*

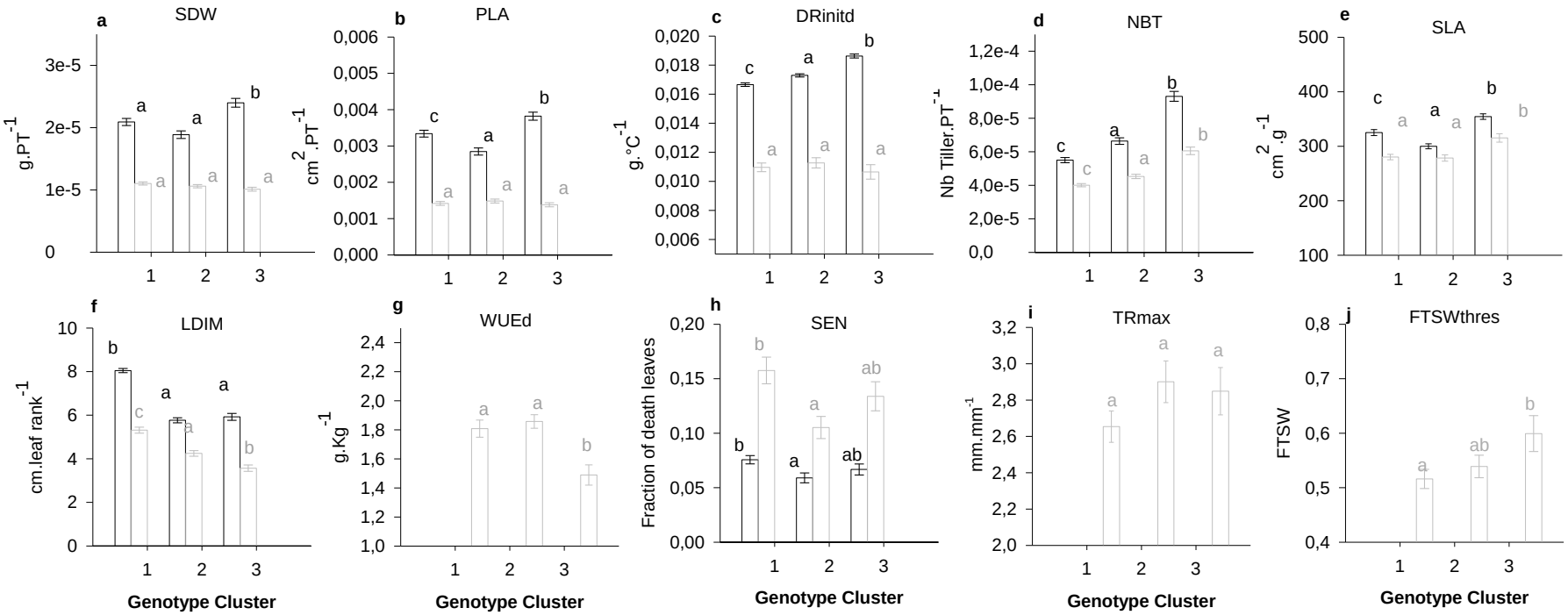

Figure 3. *Average values for morphological traits in each genotype cluster (1, 2 and 3, defined in Table 6) normalized by the photothermal time: a: Shoot Dry Weight, b: Plant Leaf Area, d: Tiller number. Variables normalized by the leaf rank: e: Specific leaf Area. f: dimensions of the last ligulated leaf. And c: Developmental Rate before stress onset, h: Fraction of Senescent leaves on the main stem, i: Maximal transpiration rate expressed as a fraction of evaporation rate, j: Thresholds for relative transpiration reduction expressed as a FTSW value. Black bars correspond to the well watered treatment and grey bars to the water deficit treatment. Standard error for each treatment and cluster are shown. Tuckey test (p<0.05) is presented by letters to show the difference between genotype clusters for each treatment (black values: well-watered, grey value: water deficit).*

DISCUSSION

The present study used a phenotyping setup to explore the diversity of tropical japonica rices for traits contributing to early vigor, performed a cluster analysis to define an empirical typology, and characterized the resulting clusters for drought tolerance (as opposed to avoidance, which was experimentally excluded) and water-use related traits. Initial hypotheses were that (i) plant growth maintenance under drought is negatively related to constitutive early vigor, (ii) this trade-off may be related to water-use related traits, and (iii) the typology based on morphological and phenological traits may bear a relationship with ecosystem adaptation, drought tolerance and breeding history. The first hypothesis was put forward by Rebolledo *et al.* (Submitted), based on a similar but independent experiment.

Rebolledo *et al.* (in press) established that the organogenetic developmental rate (DR, equal to 1/phyllochron) is a driver of early vigor in the absence of water limitation by generating a rapid succession of sinks. Luquet *et al.*(in press)confirmed this concept using a modeling approach, explaining the previous observation that vigorous genotypes have larger transitory non-structural carbohydrate (NSC) pools but Rebolledo *et al.* (Submitted) show that this genotypes are poorly drought tolerant. Finally, Rebolledo *et al. (*Submitted) attempted a typology of rice genotypes on the basis of all the above. This study confirms and extends this approach to the hypotheses described above, using independent, and comprehensive phenotyping data on vegetative plants.

Traits conveying early vigor are associated with poor drought tolerance

Traditional upland adapted rice varieties have on average broader leaves and lower tillering ability than lowland adapted varieties (Courtois *et al.* 1999). This is in agreement with the present typology, namely cluster 1 vs. 3. Under well watered conditions, DR and leaf size were negatively correlated with each other but contributed both positively to early vigor in terms of biomass and leaf area development, confirming the independent study by Rebolledo *et al.* (in press). By contrast, SLA, a trait that has been associated with early vigor in rice (Namuco *et al.* 2009) was not significantly correlated with shoot biomass and had lower heritability compared to other component traits of vigor such as DR, leaf size or tiller production. This may be due to a large phenotypic plasticity of SLA (Rebetzke *et al.* 1999; Rebetzke *et al.* 2004) and may also depend on genotype. The advantage conveyed by high SLA is the smaller physiological cost of leaf area growth during exponential growth when leaf area is limiting (Dingkuhn et al., 1999), but this advantage may not materialize in genotypes and environments where exponential growth is sink limited (Rebolledo et al.in press).

Interestingly, SLAc was positively correlated with traits setting the pace of organogenesis (DR, tillering). Regardless of whether this linkage is physiological or genetic, it may be of adaptive value

because in rapidly expanding plants, high SLAc (thin leaves) would offset some of the sink limitation. We also found SLAc to be negatively related to WUEd. This can be explained by the fact that high SLAc is associated with lower maximal leaf photosynthetic rates (Condon *et al.* 2004), while leaf conductance is not necessarily smaller. This is consistent with our observation that the reduction of SLA under drought was positively correlated with WUEd, similar to findings of (Cabuslay *et al.* 1999). An increase in leaf thickness under water deficit could be also related to greater allocation of assimilates to leaves in order to maintain plant functional equilibrium (Poorter *et al.* 2000) or may just be caused by restricted transport to, or impeded sink activity in, heterotrophic organs. Under drought, plants are probably not source limited (Rebolledo *et al.* Submitted).

Constitutive traits contributing to vigor were negatively correlated with their maintenance under drought (Table 4). However, only traits related to organogenetic processes (DR and tillering) were associated with biomass growth reduction under drought. They were also associated with low WUE during the drought cycle and with a high critical FTSW (FTSWthres). This confirms the trade-off between early vigor and growth maintenance under drought (Cabuslay *et al.* 2002; Rebolledo *et al.* Submitted).

The correlations among traits, although statistically significant, were mostly weak in terms of R values. This is not surprising, given the genetic diversity of the panel, as opposed to mono-parental mapping populations or isogenic lines. The inter-trait relationships across the population translated into clusters of genotypes reflected the trade-offs described. High-tillering cluster 3, for example, associated superior vigor with high SLA and sensitivity of transpiration to soil water deficit. Large-leafed cluster 1, which represented an opposite strategy as compared to cluster 3, achieved vigor with large organs and small organ number, while transpiration was comparatively insensitive to FTSW. But only cluster 2, which had poor vigor and small, comparatively thick leaves, was drought tolerant in terms of biomass and leaf area maintenance, while showing the least senescence under both control conditions and drought.

Further research should determine whether these trait associations are the result of ecosystem specific selection pressure and breeding history, structural genetic linkages or inherent physiological trait interactions. The present results point at a strong contribution of ecosystem adaptation and crop improvement to cluster characteristics but the available information was limited. Furthermore, the genetic composition of the panel (a large body of tropical japonica rices augmented with a small sample of accessions from the other genetic groups of the species (Perlegen sample) did not allow a meaningfull typology across the entire species.

What caused the trade-offs between early vigor and drought tolerance?

This study was designed to identify existing patterns of traits related to early vigor, drought tolerance and water use, but it permits no overall assessment of drought adaptation because avoidance mechanisms (e.g., deep root system) were excluded by the experimental design. Genotypes were submitted to the same final drought intensity and each plant transpired the same amount of water to attain FTSW' 0.2. Plants were thus not penalized for vigor, which necessarily increases water consumption (Sinclair *et al.* 2001) and for that reason alone can cause a poor drought rating in the field. The trade-off with drought tolerance observed here was thus physiological and not a matter of access to water. Our observations on water use patterns provide some new evidence beyond the findings of Rebolledo et al. (submitted). Cluster-3 genotypes, which included aquatic rices, responded to very mild drought with reduced transpiration, presumably through stomatal closure. By contrast, the mostly traditional upland rices found in cluster 1 had a lower critical FTSW. Serraj *et al.* (2008) reported similar trends but even greater genotypic differences, with FTSWthres equal to 0.34 for a traditional, tropical japonica, upland adapted cultivar (APO) and 0.73 for an improved, indica, lowland adapted cultivar (IR72). The high critical FTSW observed for many of the cluster-3 genotypes could theoretically be seen as a "cautious" response protecting the soil water reserve, and should then theoretically be associated with increased transpiration efficiency, or WUE at the crop scale, because partial stomatal closure increases transpiration efficiency (Impa *et al.* 2005; Kato *et al.* 2008; Sinclair *et al.* 2008). The opposite was the case here: cluster 3 having substantially lower WUE during the drought cycle than clusters 1 and 2. The early response of transpiration rate to decreasing FTSW was thus not an adaptation but an expression of stress, which probably affected photosynthesis not only through stomatal closure but also more directly (Araus *et al.* 2002).

Large-leafed cluster 1 had higher WUE and lower critical FTSW than cluster 3 while suffering smaller relative growth reduction under drought. Previous studies on rice also reported a better growth maintenance under drought for genotypes with higher WUE (Cabuslay *et al.* 2002; Farooq *et al.* 2010) and lower critical FTSW (Kato *et al.* 2008; Serraj *et al.* 2008). In our study, we did not measure WUE on control plants and can therefore not tell whether differences in WUE under drought were due to adaptive response or constitutively greater WUE.

The daily normalization of plant transpiration rate by leaf area PLA aimed to avoid bias through plant size. This can introduce another bias, that of variable mutual shading of leaves. We consider this bias negligible here because plants were isolated from each other (LAI in the population did not exceed 0.5). Plant architecture, namely leaf angle, can also be expected to introduce a systematic error that cannot be evaluated here. The resulting TRmax term is thus fraught with some uncertainties.

The normalization of TR with PET was necessary because transpiration is a direct function of PET. It resulted in a term resembling the Kc term of FAO (Allen et al., 1998), but attaining much larger values because it is leaf area instead of field area based, in a situation where LAI is small and therefore boundary layer resistance is small. Differences in LAI did to cause bias in TRmax estimation, as indicated by the absence of correlation between plant leaf area and TRmax.

Maximal leaf transpiration rate and critical FTSW were significantly and positively correlated, indicating that plants having constitutively high transpiration rates responded sensitively to FTSW. This trend was previously reported for pearl millet and groundnut (Kholova *et al.* 2010; Devi *et al.* 2011). Although TRmax showed high heritability in our study (0.79), its mean values did not differ among clusters. The strong genotypic differences in TRmax were probably independent of the morphological and phenological traits used to define the clusters (leaf size, DR, tillering, SLA), which were more strongly inter-related.

Genetic diversity in early vigor, water use and drought tolerance: Opportunity for breeding?

Traditional cultivars dominated large-leafed cluster 1 whereas improved materials were most frequent in the less vigorous but drought tolerant cluster 2. Tropical-japonica based breeding has thus on average caused a shift in plant type towards smaller stature, inferior early vigor and greater drought tolerance, while maintaining the relatively insensitive response of transpiration to FTSW observed in cluster 1. Aquatic indica and aromatic rice groups, only marginally represented in this study, were associated with cluster 3. Although Parent *et al.* (2010) reported that rice drought resistance was mainly improved through avoidance through deeper root systems; our study suggests that gains were also made in drought tolerance.

The ideal morphological ideotype for water limited rice systems is still a matter of debate and may depend on drought type and phenological timing. The advantage of large-leafed genotypes, showing higher WUE under drought, was previously reported for rice (Xu *et al.* 2009; Farooq *et al.* 2010). In our study, large-leafed cluster 1 had greater leaf senescence under drought than small- and comparatively thick-leafed cluster 2, involving a strong decrease in green leaf area. Leaf senescence, however, can also have adaptive value: For sorghum, Blum *et al.* (1984) reported senescence of older leaves to be associated with higher WUE enables by higher photosynthetic rates of young leaves. On the other hand, the small leaves of semi-dwarf rices tend to have greater capacity for osmotic adjustment (Dingkuhn et al. 1991) and this plant type's higher development and tillering rate is an advantage during recovery after drought (Luquet et al. 2008).

For water-limited systems, from an agronomic perspective, it would be advantageous to combine bold growth behavior (early vigor, high leaf transpiration rate, insensitivity of stomata and expansion processes to mild water deficit) with good drought avoidance (as a morphological root trait, not studied here) and good tolerance. These are composed of numerous component traits having largely unknown physiological trade-offs and genetic linkages, which should ideally be known before marker-assisted trait pyramiding is attempted on a larger scale. Furthermore, effective markers remain to be identified for most of the traits concerned, and to find the best markers, they should be extracted from the largest possible allelic diversity. Xu *et al.* (2009) reported the difficulty of finding robust QTLs for WUE in rice. Bhatnagar-Mathur *et al.* (2007), however, reported improved transpiration efficiency and growth maintenance under drought for groundnut transformed with the DREB1A gene. Major QTLs were found in maize for maintenance of leaf expansion under drought using a model-assisted phenotyping approach on the basis of a dry-down system (Tardieu *et al.* 2005).

Species or sub-species wide association genetics (McNally *et al.* 2009) on the basis of phenotyping approaches similar to those presented here, but technically further improved, may open new inroads for crop improvement.

Need for improved, high throughput phenotyping methodologies.

This study largely relied on manual plant measurements and model-assisted estimations of several key variables, such as the reconstruction of leaf area kinetics with the morphogenetic model Ecomeristem (Luquet *et al.* 2008a) on the basis of observed final tiller number, final green and senescent leaf number, shoot dry weight, and leaf dimensions; as well as observed time courses of atmospheric conditions, of water use and FTSW during drought. The uncertainties mentioned above can only be eliminated with more detailed and continuous observations on plant structural dynamics using imagery interpreted by spatial-topological models (Tardieu *et al.* 2010; Furbank *et al.* 2011). Such systems are currently under development specifically for rice at the International Rice Research Institute (IRRI, Philippines) and also in China and Brazil. A global rice phenotyping network involving large parts of the international rice research community will ensure coordinated use and improvement of such approaches (Doberman 2011).

The genotypic differences in water use related variables (maximal leaf transpiration rate, critical FTSW, WUE) and their association with morpho-phenological plant types (clusters having similar trait combinations) as observed here deserve further study. Due to technical difficulties and imprecision of measurements, only 88 out of 176 genotypes provided reliable data on critical FTSW and TR vs. FTSW slope parameters. But the available results justify further investment, including non-intrusive

monitoring of transpiration rate and photosynthetic variables at the leaf level, possibly through thermal and spectral imagery (Berger *et al.* 2010), as well as chlorophyll fluorescence imagery (Furbank *et al.* 2011). These advances will permit not only to help quantifying the trade-offs among early vigor, WUE and drought tolerance, but also identifying the physiological and genetic causes, and possibly devising strategies to overcome some of the trade-offs (Araus *et al.* 2002; Condon *et al.* 2004; Blum 2009).

Our results suggest that combining a phenotyping approach on diversity panels (as opposed to small samples of contrasting genotypes) combined with model-assisted, multi-trait analyses has the potential of combining depth (physiological component traits) with breadth (e.g., selection history, ecosystem of origin, genetic groups). And lastly, using diversity panels provides direct shortcuts to genetic analyses of traits through molecular association genetics *(Roy et al. 2011)*, potentially feeding into molecular breeding pipelines.

CONCLUSION

This study confirmed trade-offs between early vigor and drought tolerance traits on a rice panel representing the genetic diversity of the tropical japonica group, augmented with a sample of genotypes from the other *O. sativa* subspecies. Genotype clusters with characteristic trait combinations were identified that were associated with breeding history and ecosystem of origin. The multi-trait, crop model-assisted phenotyping approach proved useful to identify naturally occurring trait associations and to interpret them on a functional basis. This will be valuable for the interpretation of the genome-phenome associations that will be studied as a next step. The results call for further improvements of the multi-trait phenotyping approach, namely the inclusion of physiological traits associated with water and radiation use efficiency under control conditions and drought.

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Headlines Chapter III

- The aim of this chapter was to test on an independent data set whether drought tolerance was negatively related to early vigour within the genetic diversity of japonica rice, and to relate it to hydraulic processes, such as plant transpiration rate, its regulation (stomatal sensitivity) and efficiency (Water Use Efficiency) under drought.

- Potential plant transpiration rate was positively related to stomatal sensitivity; however no relation was found between transpiration and early vigour related traits.

- Early vigor related traits were significantly related to stomatal sensitivity: DR was significantly positive related to FTSWthres, conversely Leaf size was significantly negative related to FTSWthres.

- Constitutive sink component traits of early vigor were used to group studied genotypes: two groups with a constitutive advantage but poor drought tolerance and one group with low vigor but high tolerance to drought were identified, confirming clusters in chapter II.

- Leaf size and its maintenance were strongly related to biomass maintenance under drought. Large leaf genotypes grouped traditional upland tropical Japonica varieties with high water use efficiency but high senescence rates.

- Plants with high Development Rate showed high stomatal sensitivity and low water use efficiency under drought. Lowland irrigated, high yielding varieties were mainly associated to this behaviour.

- Water use related traits measured under drought showed a wide genetic diversity in the studied panel although they were negatively associated to early vigor. The genetic study of these traits and the possible genetic co-location should raise opportunities to increase water use efficiency through improved drought tolerance in rice without affecting early vigor under well watered conditions.

Synthesis, discussion and perspectives

SYNTHESIS

The aim of this thesis was to characterize morphogenetic traits, water use and metabolic traits **underlying the genetic and phenotypic diversity of rice early vigor (biomass accumulation) and its maintenance under drought.** A panel of *O. sativa* japonica accessions (see **Appendix II** for details) including traditional and improved genotypes with different agro-ecological adaptation was phenotyped in two pot experiments. A phenotyping dry-down approach was successfully adapted to a large number of genotypes and served to: (i) observe genotypic differences in early vigor and its maintenance under drought, (ii) identify trait associations and vigor vs. drought tolerance trade-offs and finally (iii) provide less complex component traits of vigor and drought tolerance for genetic association studies.

The present thesis aims to test the hypothesis that sink dynamics is a major driving force of resource acquisition, rate of exponential growth and thus, of early vigor (Dingkuhn *et al.* 2006; Luquet *et al.* 2008; Gerardeaux *et al.* 2011) in rice, a model plant for branching cereals. Traits related to plant morphology and phenology at organ level were addressed : Leaf size (LDIM), leaf number (LNB), Leaf appearance rate (DR), Leaf Senescence (LSEN), Tillering (NBT), as well as traits related to Non Structural Carbohydrate (NSC) pools in vegetative source and sink organs. In chapters I and II we explored to which extent rice genetic diversity in terms of early vigor and its response to drought could be explained by differences in sink dynamics (organogenetic developmental rate and tillering) and how it related to NSC pool sizes. In Chapter III differences in sink activity under drought were related to traits affecting water use, in particular plant leaf transpiration rates and its control by soil water deficit and association with the morphological phenotype (e.g., specific and plant leaf area: SLA ($cm^2.g^{-1}$) and PLA(cm^2)). Thus, in chapters II and III we concluded on the association of sink dynamics related traits with drought tolerance. These phenotypic associations provided a typology for the germplasm studied. Trait combinations occurring within each type were an expression of physiological trade-offs, and there were indications that selection or domestication environments have contributed to the phenotypic features of each group.

In chapters I, II and III, data from experiment 1 (Montpellier, Exp1) and 2 (IRRI, Exp2) were analyzed separately, although consistent results were obtained in the experiments. The present chapter aims at providing an integrative analysis and discussion of all results obtained, and makes suggestions for future studies.

DISCUSSION

Developmental Rate was identified as most important driver of early vigor

Developmental Rate (DR: 1/ Phyllochron) was demonstrated as being a major driver of early vigor, defined by Relative Growth Rate (RGR), Shoot Dry Weight (SDW) and Plant Leaf Area (PLA), during exponential growth. An analysis of the sensitivity of Ecomeristem simulations to the variation of its genotypic parameters governing source and sink dynamics confirmed that shoot biomass accumulation during exponential growth is most affected by DR under well watered conditions. Indeed, DR impact was stronger than that of any other sink related parameter such as tillering ability (the degree to which tiller outgrowth responds to assimilate availability) and potential organ size, and that of source related parameters, in particular light conversion efficiency (Epsib **Appendix I**).

In the same model assisted study, the optimization of model parameters for each of the 200 genotypes constituting the japonica diversity panel and studied in Exp1 under well watered conditions, indicated that the diversity observed in shoot biomass accumulation was explained, in decreasing order of significance of parameters participating in the step-wise multiple regression analysis, by variation of DR and Epsib. Thus, further exploring source related parameters, in particular photosynthesis related traits, will improve the understanding of genotypic diversity of constitutive early vigor.

New phenotyping platforms and association genetics will answer questions on early vigor

We could not check through direct measurements whether DR and other sink related parameters explained early vigor better than variation in source related traits. Gu *et al.* (2012) reported QTLs for photosynthetic traits (i.e. photosynthetic rate, stomatal conductance, transpiration rate, quantum yield of photosystem II) both under well watered and water deficit conditions. Thus, it would be worth quantifying for the panel studied here the genetic variation for traits related to C assimilation, including dark respiration. Chlorophyll florescence analysis using excitation kinetics of photosystem II with very high temporal resolution (e.g., Handy Pea, Hansatech Instruments ltd, Norfolk, UK) or fluorescence remote sensing under controlled light conditions on specialized platforms (e.g.,Furbank *et al.* (2011) are suited for high-throughput (HTP) phenotyping of photosynthetic efficiency and many complementary plant traits for a large number of genotypes. This technology should be used in future research project following GenPhen and Orytage, to which this thesis has contributed. In the context of GRiSP (Global Rice Science Partnership) of the CGIAR (Consultative Group of International Agricultural Research), a Global Rice Phenotyping Network was initiated in 2011 (http://grisp.irri.org/theme-1-documents). Specifically, GRiSP Frontier Research Project PRAY

(Phenomics of Rice for Adaptation and Yield potential) was accepted for funding in early 2012 and will answer many of the questions brought up by this thesis.

Sink related variables DR, LDIM and NBT exhibited greater broad-sense heritability than SDW in both experiments (Table 1). These traits as well as Ecomeristem fitted parameters will undergo genetic association analyses using a 1-million Single Nucletide Polymorphism (SNP) chip currently under development by a consortium including Cornell University, IRRI, CIRAD and other partners. The association study will answer several of the questions raised in this thesis, for example:

- Which phenotypic traits that appear to be linked, or occur in combination on the clusters of genotypes defined here, give co-localized signals on the rice genome?
- Do parameters directly measured or obtained through model fitting give stronger association signals on the genome? Do they associate with identical or different loci?
- Consequently, is phenotyping assisted by crop models such as Ecomeristem suited for HTP and does it provide added value?

One advantage of model assisted phenotyping is the possibility to use the model as predictive tool. Ecomeristem can thus be used to explore ideotypes, i.e. define optimal combination(s) of traits (parameter values) for the expression of early vigor in different environments. Optimal trait combinations should indeed vary depending on the TPE and the interaction with other source-sink related traits not measured in this study, and the corresponding parameters in Ecomeristem not analyzed here (e.g., roots, respiration)(Sinclair 2011)

Table 1. Mean values, standard deviation (Sd), variation coefficient (CV) and broad sense heritabilities (H^2) for component traits measured in experiments Exp 1 (Montpellier) and Exp 2 (IRRI) on well watered (c) plants: NBT (Normalized tiller number normalized by photothermal time), NBL (number of ligulated leaves on the main stem normalized by the photothermal time), DRinits (Developmental Rate before stress onset of well watered plants), SDW (shoot dry weight (in g) at the end of the treatment normalized by photothermal time). Different letters indicate significant differences identified by a student test between environments (p<0.05) (see Chapter I and III for details on variables.

Variables	Mean	Sd	Cv	H^2
NBTc_exp1	4,509E-05	1,483E-05	0,33b	**0,44**
NBTc_exp2	6,948E-05	2,258E-05	0,32a	**0,84**
LDIMc_exp1	5,299	1,335	0,25b	**0,54**
LDIMc_exp2	6,751	1,424	0,21a	**0,84**
NBLc_exp1	1,365E-04	3,320E-05	0,24b	0,46
NBLc_exp2	1,879E-04	5,287E-05	0,28a	0,84
DRinitc_exp1	1,588E-02	1,304E-03	0,08b	**0,66**
DRinitc_exp2	1,733E-02	1,353E-03	0,07a	**0,86**
SDWc_exp1	1,495E-05	4,008E-06	0,26b	0,08
SDWc_exp2	2,120E-05	5,158E-06	0,24a	0,52

Developmental rate, tiller number and leaf size: physiological or genetic trade-offs?

Our experiments showed two interesting physiological linkages under well watered conditions. Firstly, chapters I, II, and III showed a positive linkage among DR, tillering rate and SLA (chapter III). Sugar content analyses provided a functional description of this linkage and suggested that early vigor related to high tillering, DR and SLA was associated with low starch storage in leaves sampled at the end of the night (reflecting the amount of starch not used for growth). This behavior was associated with the production of leaves at comparatively low carbon cost (high SLA).

Secondly, a negative correlation between DR and tillering rate with leaf size (LDIM) was observed in both experiments and also linked to plant NSC status. In fact, in the subsample studied in chapter II as well as in Ecomeristem simulations (**Appendix I**), genotypes characterized by high DR exhausted daily starch reserves to the benefit of a rapid growth, whereas large-leaf genotypes having slower development tended to store starch. Consequently, cluster analysis in chapters II and III showed that early vigor was related either to large leaf size or to high DR.

Consequently, as described in chapter II, starch content in source and sink leaves was indicative of early vigor by two different ways (**Figure 1**) :(i) s**ource limited (sink driven), "bold" plants,** with high DR and tillering rate but small leaves and exhaustive use of assimilates for growth (as indicated by low starch storage) and (ii) **large leaf, sink limited (source driven), "conservative" plants** with high starch storage in source and sink leaves. Conservative plants were characterized by a low sink C demand, which would suggest:

- A positive relation between growth and starch availability: in this case plants should be defined as "source driven"
- A potential to further increase vigor by increasing DR through breeding: in this case, this type of plants should be defined as "sink limited".

In *Arabidopsis*, sink driven and source driven behaviors were suggested: Sulpice *et al.* (2009) showed that plants with higher growth rate have better C use efficiency than plants accumulating starch. However, Gibson *et al.* (2011) studied rice and *Arabidopsis* mutants with enhanced capacity to accumulate starch in source leaves and showed that C storage as a transient sink enhances photosynthetic capacity and thus biomass accumulation. In the latter case, it is an open question whether increased starch accumulation was a result of reduced demand for C (which is unlike, given the increased growth rate) or of enhanced assimilation rates.

Finally, the functional linkage pointed out in the present study and reports for co-location of QTL for growth traits and NSC concentrations in Arabidopsis (Sulpice *et al.* 2009) suggest that the analysis of tissue sugar contents should be further pursued. Only a subset of the panel was analyzed for this trait in Exp. 1, and analyses should be completed for the whole diversity panel. This will provide further insight into the physiological linkage between NSC and early vigor patterns and enable introducing metabolic traits in the genetic association studies planned for 2012. It will also be possible to explore whether or not the linkages and trade-off between DR, leaf size and NSC use or storage have a genetic bases and consequently, to what genetic linkages may have to be overcome in the quest to improve early vigor.

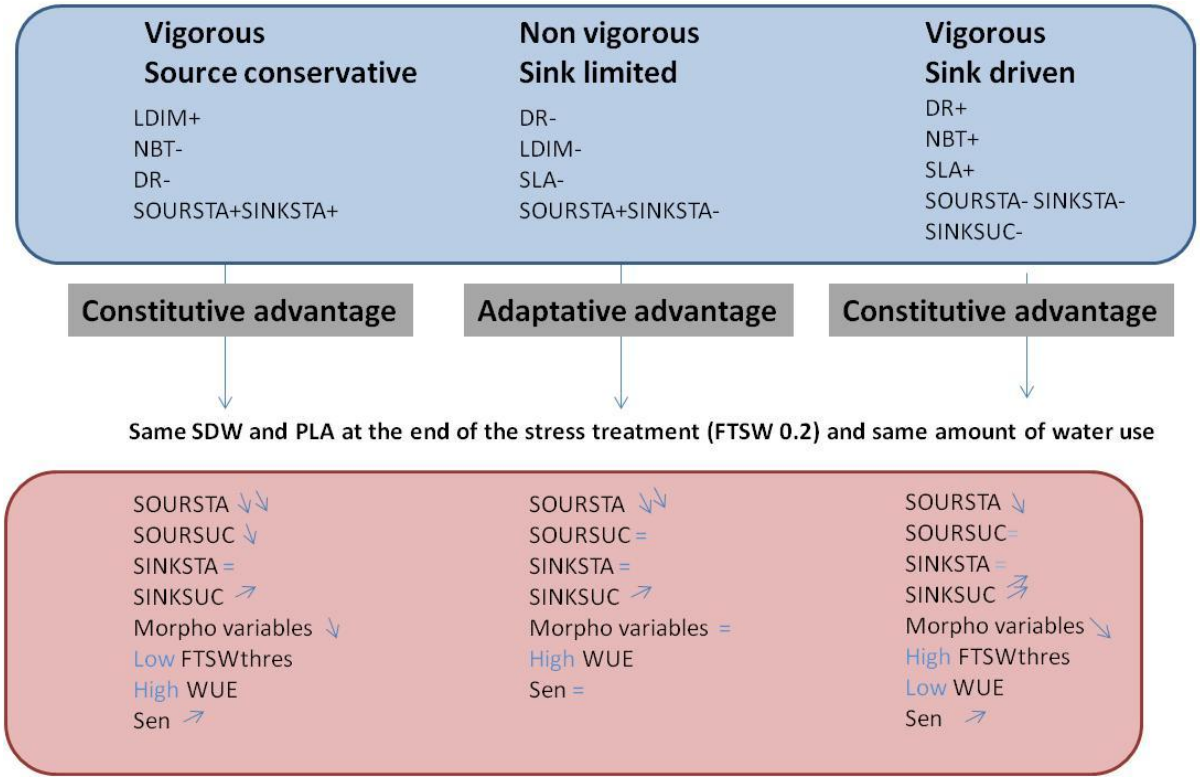

***Figure 1**. Conceptual scheme (from results in Table 7 of Chapter II, and Table 5 of Chapter III) representing early growth patterns in the japonica rice diversity panel studied under well watered conditions (blue) and their advantage (in grey) on growth and water use under drought conditions (red); extrapolated from the combination of results from chapters I, II, III. Variables are detailed in these chapters. In the blue zone signs represent variables with higher (+) or lower (-) values, in the red zone the direction of the response variables (dc/c) are represented (increase, decrease, and stable).*

Does sink strength limit biomass accumulation under drought?

In both experiments, DR under drought showed low broad-sense heritability (Table 2) and lower correlation with biomass growth maintenance, as compared other sink related traits (chapters II and III). The low impact of DR under drought on growth maintenance might be explained by:

- On average across experiments and genotypes, only two leaves appeared during the drought treatment. It is known that under well watered conditions, four leaf primordia (initiated at the rhythm of plastochron) grow simultaneously in rice, hidden in the sheaths (Itoh *et al.* 1998). Therefore, leaves appeared during the drought treatment had been initiated under well watered conditions prior to stress onset, when plastochron was not yet affected by drought. As DR is the combined result of plastochron and leaf expansion rates its observed reduction under drought was the result of reduced leaf expansion rate and not of reduced development.

- Depending on the genotype, phyllochron may also increase (or DR decrease) around the 8-10th leaf stage on the main stem, roughly at the same time of panicle initiation and the onset of internode expansion (Lafarge *et al.* 2010). This might have affected the observations for DR during the drought treatment in the present study.

To further explore the impact of DR on rice biomass production under drought, it would be interesting to increase the duration of the dry-down treatment by managing irrigation in a way that maintains a constant stress level during the time needed for the appearance of more than 4 leaves on the main stem. The new version of *Ecomeristem* adapted to drought situations(Luquet *et al.* 2008a) will be applied to explore to what extent DR (genotypic parameter of the model) and its regulation by drought (through effects on both leaf initiation and expansion rate) can be a favourable or undesirable trait, depending on the drought type (for example a slow, long dry-down vs. a rapid severe stress). This planned research was inspired by this thesis project and will be conducted as part of the exploration of improved ideotype concepts.

Drought tolerance is related to the maintenance of organ function and growth, particularly leaf size. Leaf size maintenance under drought had the highest broad-sense heritability (Table 2) in both experiments and was significantly related to the maintenance of plant green leaf area in Exp2. Final leaf size depends on cell division and expansion rates at meristem level. Because of the short duration of the drought treatment in our study, leaf size reduction during the dry-down cycle was probably due to a reduction of cell expansion, and to a much lesser extent to inhibited division rates that occur at an earlier stage of leaf development, as it was observed on maize (Tardieu *et al.* 2000). The high sensitivity of leaf size to drought (Chapter II and III) was also reported in other studies on maize (Chenu *et al.* 2008) and rice (Luquet *et al.* 2008; Parent *et al.* 2010). Indeed, rice genotypes showed a strong diversity in terms of leaf size and its sensitivity to drought in our study (Chapters II and III). Therefore it would be interesting to characterize for the genetic basis of leaf expansion rate response to drought in the japonica rice sub-species, as performed by Raymond *et al.* (2004) on maize mapping populations.

Table 2. Mean values, standard deviation (Sd), variation coefficient (CV) and broad sense heritabilities (H^2) for component traits measured in experiments Exp 1 (Montpellier) and Exp 2 (IRRI) on stressed plants : DRonset (Developmental rate from stress onset to the end of the treatment; °Cd^{-1}), NBT (Normalized tiller number normalized by photothermal time), NBL (number of ligulated leaves on the main stem normalized by the photothermal time), DRinitS (Developmental Rate before stress onset on future stressed plants), SDW (shoot dry weight (g) at the end of the treatment normalized by photothermal time). Different letters indicate the significant differences between environments identified by a student test (p<0.05) (see Chapter II and III for details on variables).

Stress Plants	Mean	Sd	Cv	H^2
DRonsets_Exp1	1,325E-02	2,513E-03	0,189b	0
DRonsets_Exp2	1,096E-02	2,867E-03	0,261a	0,21
NBTs_Exp1	4,102E-05	1,238E-05	0,300b	0,26
NBTs_Exp2	4,759E-05	1,412E-05	0,295a	0,75
LDIMs_Exp1	4,639	1,126	0,242a	0,54
LDIMs_Exp2	4,488	1,295	0,287a	0,75
NBLs_Exp1	1,253E-04	3,093E-05	0,2460a	0,32
NBLs_Exp2	1,313E-04	3,232E-05	0,245a	0,75
SDWs_Exp1	1,199E-05	2,782E-06	0,231b	0,05
SDWs_Exp2	1,064E-05	2,085E-06	0,195a	0,68
DRinitS_Exp1	0,0158	0,0013	0,0838b	0,63
DRinitS_Exp2	0,0174	0,0013	0,0718a	0,86

Three pathways to attain the same biomass after a drought treatment: Opportunities to improve early vigor for drought prone environments?

The correlations observed between metabolic (NSC) and morphogenetic traits under drought were not highly significant *(pvalues<0.01)* (Chapter II). As suggested in other studies (Sulpice *et al.* 2009; Stitt *et al.* 2010), multivariate analysis including principal component analysis (PCA) can help identifying how metabolic and morphogenetic traits are combined.

The PCA and clustering performed in chapters II (on 43 genotypes in Exp1) and III (on 176 genotypes in Exp2) pointed out that drought tolerance, i.e. plant growth maintenance, was related to low constitutive biomass accumulation under well watered conditions. However, as constitutive vigor enabled a higher biomass accumulation before stress, shoot biomass present at the end of the stress period for stressed plants was not significantly different among clusters (Chapter II, III, and **Figure 1**).

In this study, growth maintenance was not related to a reduction of water use (water conservation), as commonly associated with low-vigor plants (Blum 2009). The drought tolerant group (low vigor

under well watered conditions and high biomass maintenance under drought, represented by Cluster 3 in chapter II and cluster 2 in chapter III) combined maintenance of growth and development with low senescence, high WUE under drought, and high starch content in source leaves under well watered conditions.According to McDowell (2011), plants use stored NSC for the maintenance of cell survival and functioning, through respiratory metabolism and osmotic adjustment. Plants that are drought tolerant, in the sense of superior maintenance of cell functioning at a given level of water deficit, are able to maintain stomatal regulation while protecting the photosynthetic system from damage by reactive oxygen species (ROS) (Farooq *et al.* 2009), resulting in higher transpiration efficiency. Future physiological studies targeting the hydraulic behaviour of "low vigor" genotypes should help to determine to what extent and how the hydraulic behaviour is associated with leaf size, DR and leaf thickness. The feasibility of co-selecting for large leaves and/or high DR with traits related to drought tolerance should be explored.

Early vigor defined by high DR and tillering (sink driven vigor) is an advantage for rapid growth under well watered conditions and for rapid recovery after a severe drought event (Luquet *et al.* 2008). However it was related to strong growth reduction under drought due to (i) low starch accumulation under well watered conditions, probably limiting its mobilization under drought to maintain tillering and (ii) a sensitive response of transpiration rate to FTSW, probably due to high stomatal sensitivity. The latter was associated with low WUE, indicating that the photosynthetic apparatus itself was also sensitive to drought. (Stomatal closure alone increases transpiration efficiency.) High stomatal sensitivity in plants to drought is not necessarily due to a sensitive response by stomata themselves, but can also be caused indirectly by stress effects on other processes such as failure of the photosynthetic system (e.g., photoinhibition, causing a rise in Ci which in turn closes stomata), xylem cavitations (causing leaf dehydration) and root dysfunction, exposing the plant to greater risks of both assimilate and water starvation (McDowell *et al.* 2008).

Therefore, future research on rice early vigor and drought tolerance should explore the feasibility to combine:

- **High DR and large leaves**, if larger leaves enable increasing leaf starch accumulation prior to stress (Chapter II), representing a potential NSC buffer for fluctuations in assimilation rates, for example, under drought. The potential advantage of high DR resides on accelerated growth recovery due to rapid organ development after a stress event.

- **Large leaves and low senescence**: large-leaf genotypes had high senescence rates under drought (Chapter II and III), thus increasing loss of biomass. Further studies on the regulation of leaf senescence in relation with leaf size will help determining if tissue anatomical traits in

large leaves penalize the maintenance of cell and tissue functioning under drought. If this is the case, the linkage between leaf size and senescence will have to be overcome to enable co-selection of these traits.

- **Early vigor, high transpiration rates under well-watered conditions and biomass maintenance under drought.** Sensitivity of transpiration rate to FTSW (FTSWtrhes) was correlated with constitutive DR, leaf size and maximal transpiration rate. However, no relationship was found between maximal transpiration rate and DR or leaf size (chapter III). Thus, more research is needed to understand the linkage between high maximal transpiration rate, early vigor and sensitive drought response of transpiration rate. Processes not studied in this thesis should be considered, such as source related traits (namely, photosynthetic processes and factors for leaf conductance such as stomatal density), oxidative stress, sensitivity to cavitation and its anatomical causes, root radial conductance and other traits that affect the conductance of water in the plant.

- **Drought tolerance during vegetative vs. reproductive growth stages**. A positive relationship between vegetative and reproductive stage drought tolerance was previously demonstrated for pearl millet (Kholova *et al.* 2010). If also true for rice, this might enable improving drought tolerance at the reproductive stage by selecting for young plants (Richards *et al.* 2002). Genotypic patterns for drought tolerance may be quite different for reproductive stage because of the specific reproductive processes involved.

Lastly, it must be emphasized here that this study only considered drought tolerance and not avoidance or temporal escape mechanisms. Avoidance (e.g., through deep roots) and escape (e.g., through locally adapted phenology) are essential components of any complete adaptation strategy for water-limited environments. It will be of great interest to breeders, geneticists and functional ecologists to know how the incorporation of avoidance and escape traits will affect the genotype typologies elaborated here.

Opportunities to explain natural trait combinations using a diversity panel

Because mapping populations are based on a single pair of parents, they do not represent a genetic group's allelic diversity (diversity of polymorphisms), and may not differ at all on many loci on the genome. Mapping populations thus serve to locate and characterize loci that are relevant to specific traits for which the parents differ. The phenotypic expression of such loci can then be studied in detail, for example by developing Chromosome Segment Substitution Lines (CSSL).

Diversity panels can also serve the purpose of gene and allele discovery by association study, but on the basis of large genetic diversity and on the basis of the natural (or breeding-induced) range of trait combinations found on the panel. Phenotyping diversity panels, as the one addressed in the present work, can answer questions related to groups having common agro-ecological adaptation, common domestication history or conditions, or breeding history (Weigel 2012). This advantage is at the same time a constraint in association genetics because natural genetic groups nested in the panel cause structure, as opposed to arbitrary distribution of polymorphisms, and lead to false positives in the phenome-genome association analysis. It is therefore important to characterize the structure of diversity panels both genetically and phenotypically. Such exercises, as conducted here on the phenotypic side, can also provide insights into domestication history, adaptation strategies and even functional information.

In chapters II (Exp1) and III (Exp2), constitutive and response variables for morphological traits led in both cases to similar clusters of genotypes, although the clustering was not performed with the same variables and the same number of genotypes (only 43 genotypes in Exp. 1). In order to integrate both experiments, a cross-cutting cluster analysis was performed using only constitutive component traits for early vigor (leaf size LDIM, DR and tillering NBT). Variables were grouped similarly as before in three clusters that described two vigorous groups: one with large leaves, the other with high DR, and one low-vigor group. Although groups had similar characteristics (ie.variables grouped in the same way in both experiments), only 50% of the genotypes were classified in the same cluster between experiments suggesting the presence of GxE interactions between environments for the variables measured. We were not able to further interpret the cross-cutting cluster analysis in terms of agro-ecological adaptation, and the number of common variables in both experiments was probably too small to pursue this further.

Sink related variables having a wide phenotypic variation such as LDIM, DR, NBT, and starch content in source leaves, maximal transpiration rate, critical FTSW for transpiration rate, as well as WUE will be used for genetic analyses to detect phenome-genome associations. To our knowledge, this is the first such study on early vigor and NSC of rice under drought. The results will feed into the search for and development of molecular markers for genomic selection and will certainly trigger further physiological and genetic studies on desirable traits for rice crop improvement.

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General conclusion

This PhD work demonstrated for a tropical japonica rice diversity panel that morphogenetic and metabolic traits (namely, transient NSC pools) related to sink activity are major drivers of early vigor under well watered conditions but are associated with low drought tolerance (i.e. growth maintenance under drought). Typologies of frequently occurring trait combinations were established and related to ecosystem of origin and adaptation strategies. It was also demonstrated that rice has a great genetic diversity in terms of drought tolerance traits. The results are relevant to rice crop imrovement and will directly feed into genetic association studies. It must be noted, however, that the present focus on drought tolerance mechanisms must be extended to avoidance and escape mechanisms, as well as to reproductive growth processes, in order to define complete adaptation strategies.

In order to improve rice early vigor in combination with drought tolerance, vigor component traits such as leaf size, development rate and tillering rate must be combined in a way that optimizes C and water use under well watered and drought conditions. The strong trade-offs between vigor and tolerance may be minimized through appropriate trait composition. This will require characterizing the genetic linkages underlying the trade-offs, and distinguishing them from physiological trade-offs that may be more difficult to overcome.

As a next step, genetic association studies will be perfomed in 2012/2013 after genotyping the population with a whole-genome, saturating, 1 M SNP chip. Other follow-up research to this thesis will include the use of plant growth modeling to simulate the trade-offs among adaption traits and the GxE interactions observed on the tropical japonica rice panel. Finally, the analytical approaches developed in this thesis will be extended in the context of the new GRiSP Global Rice Phenotyping Network to avoidance, escape and yield potential. This enlarged analysis will also include existing phenomics data on the tropical japonica panel obtained by the ORYTAGE project under controlled conditions (root architecture, cardinal temperatures for phenology) and in the field (Carbon isotope discrimination and infrared thermography).

Appendices

Appendix I Article Luquet et al 2011.

Submitted and accepted by journal of Agronomy and Crop science, as a complementary study (second part) of Chapter I in this work.

ORIGINAL PAPER: Developmental dynamics and early growth vigour in rice 2. Modelling genetic diversity using Ecomeristem

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ABSTRACT

Early vigour is an important objective in rice breeding. A previous study reported strong positive effects of developmental rate (DR, 1/phyllochron) on early growth vigour in two rice panels. This study provided a model based analysis of DR effects on rice early vigour and underlying source-sink processes during exponential growth, using Ecomeristem model.

Relevant model parameters were fitted to panel observations and their effect on early vigour was quantified. A sensitivity analysis was performed to quantify the impact of model parameters on simulation outputs. The simulated behaviour of a population of virtual genotypes defined by the combination of model parameter values was compared with that of diversity panel. Finally, a simulation experiment was conducted to analyse source-sink adjustments constituting early vigour across a range of DR.

Parameters governing structural development, particularly DR, had greater impact on vigour than parameters for resource acquisition. High DR was associated with rapid dry weight accumulation and low transitory carbohydrate reserves in both real and virtual populations.

Genotypic DR is thus a major driver of early vigour in rice under stress free conditions. To evaluate traits contributing to vigour, the capacity of crop models to simulate interactions between structural development and resource acquisition must be improved.

INTRODUCTION

Early vigour is an important property in annual and therefore fast-growing crops, particularly in the presence of weed competition or when the season is short. Early vigour, in terms of rapid development of biomass, leaf area and ground cover, is an emergent property resulting from many processes including resource acquisition and conversion, organo- and morphogenetic dynamics, and plant and canopy architecture.

Common crop modelling systems such as DSSAT (Jones *et al.* 2003), STICS (Brisson *et al.* 1998), APSIM (Wang *et al.* 2002) or SARRAH (Heinemann *et al.* 2008) emphasize resource acquisition and partitioning among organ classes as main drivers of early vigour, the outcome thus being mainly resource driven. Leaf area development is thereby largely driven by light interception and limited by nitrogen (N) uptake where it is considered. The degree of areal spreading of resources for leaf growth thereby is a function of specific leaf area (SLA; Dingkuhn *et al.* 2003; Drenth *et al.* 1994), or of specific leaf N (SLN) in the case of APSIM. SLA and SLN are essentially allometric coefficients setting a target value for areal spread of a resource (carbohydrates, C, or N), under the assumption that the organogenetic process can always structurally accommodate this expansion. Crop canopy architecture is usually summarily set by a genotypic and/or development stage dependent extinction coefficient K related to mean leaf inclination and independent of environment.

While these models are robust in predicting crop yield in a given type of environment, their crop parameters tend to be quite environment specific. This may be in part because (1) the conversion efficiency at which newly acquired assimilates are converted into growth is considered independent of demand; and (2) structural development in terms of organo- and morphogenetic processes, which have strong genotype x environment interactions (GxE), are considered non-limiting for biomass growth (Dingkuhn 1996; Hammer *et al.* 2002).

In the last decade, crop models were increasingly called upon to simulate genotypic differences and GxE or GxExM (M for management) (Asseng *et al.* 2002; Boote *et al.* 2003; Chapman *et al.* 2003; Hoogenboom and White 2003). Such applications even include extrapolation to hypothetical environments associated with climate change. These objectives, involving virtual genotypes in virtual environments, require improving crop models to achieve 1) greater validity across wide ranges of existing environments, 2) simulation of environment-driven phenotypic plasticity for key traits and trait combinations (Dingkuhn *et al.* 2005; Hammer *et al.* 2005) and 3) the possibility to relate model parameters to the effect of alleles on given loci or genes controlling key

traits (Chapman *et al.* 2003; Cooper *et al.* 2005). These considerations also apply to the issue of early vigour (Dingkuhn *et al.* 2005; Drouet and Pagès 2003; Yan *et al.* 2004; Yin *et al.* 2003).

To meet these new modelling needs, some crop models were coupled with models simulating morphogenetic or physiological process based traits. For example, APSIM was implemented with functions describing leaf expansion rate (Chenu *et al.* 2007), stay green traits or osmotic adjustment (Chapman *et al.* 2003) that can be linked to QTL effects. This approach, however, has its limitations as it implies that the crop model sufficiently considers physiological feedbacks at different biological scales, i.e. crop resource status, elemental organ development and plant architectural dynamics (Granier and Tardieu 2009).

Functional Structural Plant Models (FSPM), linking physiology with architectural development, are increasingly becoming important in crop research (Hemmerling *et al.* 2008; Pradal *et al.* 2008; Yan *et al.* 2004). FSPMs simulate feedbacks between resource acquisition and plant morphogenesis. Some FSPM consider organ morphogenetic processes on the basis of organ initiation and expansion laws that are modulated by carbon resources. Sinks are thus initiated and dimensioned under the influence of competition relationships among organs in the plant and among plants in the population; these regulations are a major source of phenotypic plasticity and thus of GxExM (rice: Ecomeristem, Luquet *et al.* 2006; maize: GRAAL, Drouet and Pagès 2003; many species: Greenlab, Jullien *et al.* 2010).

Depending on the model algorithms, growth simulated by FSPMs can be entirely source driven (sink strengths being relative, with no upper limit to resulting organ size; Yan *et al.* 2004) or sink driven (sink strengths are finite and pre-dimensioned at organ initiation under the influence of environment; Xu *et al.* 2011). The latter is true for Ecomeristem, a model providing for several adjustment processes to sink-source imbalances, including: organ potential size, a transitory reserve buffer, facultative organ initiation and senescence, and inhibition of carbon assimilation if the system is extremely sink-limited. According to this concept, variation of early vigour is strongly affected not only by resources *per se* but also by genotype dependent reaction norms of meristem activity to available resources. Such relationships have been experimentally observed (Beemster and Masle 1996; Fiorani *et al.* 2000; Granier and Tardieu 1999).

In the companion paper, Rebolledo *et al.* (2011) provided evidence that DR (here defined as organogenetic developmental rate, 1/phyllochron) is a key genotypic driver of early growth vigour in rice. This result was experimentally obtained from phenotyping of DR, tiller production, leaf dimensions, shoot dry weight (SDW) and relative growth rate (RGR) on two 203-strong rice panels representing rice genetic diversity (*O. sativa* L. and its tropical japonica sub-group). DR was strongly,

positively correlated with vigour parameters, and particularly RGR. Leaf size and tiller production also affected vigour positively. Leaf size was negatively correlated with both tillering and DR. Furthermore it was demonstrated that within subsamples of genotypes having similar tiller number and leaf size, the effect of DR was conserved, suggesting a direct effect of DR on early vigour. Behind these correlations are complex physiological source-sink interactions affecting the traits constituting early vigour. Their physiological and genetic components are difficult to unravel experimentally (W. ter Steege *et al.* 2005; Tisné *et al.* 2008).

The present paper's objective is to explore by modeling the morpho-physiological mechanisms and feedbacks underlying early vigour of rice seedlings. Specifically, it aims at understanding the C assimilate source-sink relationships underlying effects of DR on early vigour and its trade-offs with other components of early vigour, using data provided by Rebolledo *et al.* (2011).

The FSPM Ecomeristem was used for its capacity to simulate the elemental source-sink processes and related GxE underlying genotypic variability in rice early vigour (Ahmadi *et al.* 2008; Dingkuhn *et al.* 2006; Luquet *et al.* 2006; Luquet *et al.* 2007). The approach consists of optimizing model parameters for each genotype of the japonica rice panel phenotyped by Rebolledo *et al.* (2011). This parameterization enables quantifying i) parameter correlations across the panel's diversity and ii) their effects on early vigour as an emergent property (SDW, RGR). As a second step, a sensitivity analysis of model outputs to parameter variation is performed to characterize the physiological effects and interactions of DR on other component traits of early vigour, including leaf senescence and transitory assimilate reserves.

MATERIAL AND METHODS

Ecomeristem model

Ecomeristem is a deterministic crop model simulating plant morphogenesis at organ level at daily time step using weather and soil hydrological properties as input. The plant is simulated as an average individual of a population forming a canopy. Plant organogenetic and morphogenetic processes are driven by incremental, aggregate C assimilate source (gross photosynthesis as a function of light interception and a genotypic conversion efficiency, minus maintenance respiration) and sink (demand for current organ growth, depending on genotypic parameters (source or sink related) and environmental conditions. The model version used here simulates only the vegetative growth phase of rice. It was described in detail by Luquet *et al.* (2006). Only the principal concepts and parameters are described in the following.

a- Organ initiation rate is scheduled by a potential plastochron (*Plasto,* genotypic parameter). It is equal to the phyllochron and to the duration of rapid expansion of a leaf to expand once it has appeared (a relationship specific to rice). Once initiated, an organ *n* is pre-dimensioned (as opposed to size determination during growth), dependent on the final size (length, width) of the previous leaf *n-1*, a state variable Ic measuring the current resource situation (aggregate supply / demand), and a genotypic parameter, *MGR* (Meristem Growth Rate). *MGR* is an additive coefficient applied to the final size of leaf *(n-1)* for computing the potential size of leaf *n*. Once pre-dimensioned, a leaf expands at a Leaf Expansion Rate (LER, $cm^2\ °d^{-1}$) equal to the ratio between potential final leaf length and expansion duration (equal to *Plasto*, or longer if the source is limiting). The sum of daily potential organ expansions on the plant results in a daily demand for carbon. Areal expansion is translated into structural dry weight demand using a leaf rank dependent value of structural SLA that does not include non-structural reserves (Luquet *et al,* 2006). The same rule is true for the plant's first leaf, except that its potential properties are defined by genotypic parameters (final length and SLA) because the 1^{st} leaf is pre-formed in the embryo and is known to be genotypic (Rebetzke *et al.* 2007). (The model considers as 1^{st} leaf the first leaf having a differentiated blade and sheath, which is not the case for the prophyll) Accordingly, simulated plant SLA including reserves depends on structural SLA that varies with leaf rank and non-structural carbohydrates, assumed to be homogeneously partitioned between blades and sheaths. Potential root growth rate on day *i* is proportional to shoot growth rate on day (*i-1*), using an empirical function decreasing exponentially from germination until flowering (Luquet *et al.* 2007).

b- A plant internal competition index Ic is computed daily as the ratio $Ic=SD^{-1}$ with S (supply) being aggregate C supply and D (demand) being daily aggregate demand for organ growth, based on linear expansion kinetics. Depending on Ic, potential organ number and size is determined whenever a new plastochron begins:

- If Ic < 1, the potential size of the new organs being initiated at that time is adjusted downwards; reserves are mobilized for expanding organs; if deficit persists, the oldest leaf of each tiller is 'killed', followed by reallocation of some of their biomass; and finally, if deficit still persists, organ expansion is delayed, resulting in a longer phyllochron and plastochron.
- If Ic < *Ict* (*Ict being a Ic* threshold value for tillering, a genotypic parameter) then new tillers are not created; otherwise, the potential number of tillers is created (Luquet *et al.* 2007).
- If Ic >=1 1, excess assimilate is stored in a transitory reserve compartment or, if the storage compartment is saturated, the assimilation rate is reduced (proxy for end product inhibition).

Ic can be considered as a proxy for sugar signalling to the meristem (Rolland *et al.* 2006).

c- Environment, carbon and water balance: Ecomeristem's soil water balance and 3D computation of plant light interception were disabled in this study for simplicity and focusing on the basic morphogenetic and source related parameters of the model. Light interception was computed using a big-leaf concept and leaf area index on the basis of the Beer-Lambert law, followed by calculation of plant net CO_2 assimilation with a coefficient for radiation use efficiency (Dingkuhn *et al.* 2003).

d- Programming environment: Ecomeristem is running on the Ecophen modelling and simulation platform implemented using Delphi 6 (Kimmel 2001). Ecophen is
interfaced with the R environment (R Development 2005) to couple model execution with statistical tools, in particular sensitivity analysis and parameter
estimation procedures.

Parameter optimization

Ecomeristem parameters are estimated using the RGenoud package (Mebane *et al.* 2009) of the R software. RGenoud uses a hybrid optimization approach. As a first step it uses an evolutionary search algorithm to reduce the cost function by adjusting values for each parameter considered. As a second step (according to a shifting fitness value) classical derivative-based methods (Newton or quasi-Newton) are used that are computationally less time consuming. RGenoud can solve problems related to nonlinear and discontinuous functions, which occur in Ecomeristem.

In this study, four parameters having major effects on simulation outcome (*Plasto*, *Ict, MGR, Epsib*) were optimized for each of the 203 japonica genotypes studied in the Experiment 2 (Exp2) reported by Rebolledo *et al.* (2011). Other model parameters were fixed at rice specific values (Luquet *et al.* 2006). The two replications of Exp2 (which differ in environment because implemented successively) were considered together through multi-fitting to optimize a unique set of parameters for each genotype. RGenoud was used to fit simulations to observations by minimizing an objective cost function (Eq.1) comparing simulated and observed values at final sampling (SDW, NbT, LN_MS, LN, LA for leaf area; refer to Rebolledo *et al.* (2011) for details).

$$\mathrm{Fo} = \sum\left(\mathrm{abs}\left(\frac{(\mathrm{vsim} - \mathrm{vobs})}{\mathrm{vobs}}\right)\right) \qquad \text{Eq.1}$$

In Eq.1 *vsim* and *vobs* are respectively the simulated and observed values of variables considered for model fitting process. 'abs' indicates that the absolute value of the relative deviation is considered.

Some model parameters were parameterized directly from observation, such as initial values on first leaf length and grain dry weight as observed on each genotype.

Model sensitivity analysis

A global sensitivity analysis of the model to its genotypic parameters was performed using the Fourier Amplitude Sensitivity Test (FAST; Saltelli *et al.* 1999). This method was chosen because of its model independent property, i.e. the analytic structure of the model can be unknown. This variance-based method gives first-order and total-order sensitivity indices that quantify the influence of the variation of the input factors (parameters) on the output variations (simulated variables). The first-order indices give the share of variation directly linked to each input factor and the total indices give the share of variation related to each input factor including direct effect and its interaction with other input factors. The sensitivity index of each factor is evaluated under varying levels of all other factors considered.

Data for model sensitivity analyses and simulation experiments

An input data file for simulation experiments was generated from replication 2 of Exp2 as described by Rebolledo *et al.* (2011), including daily meteorological data (mean. air temperature of 26°C , mean PAR 5 $MJ.m^{-2}$). Plant population density parameter was fixed at 30 m^{-2}. Data for water balance computation (potential evapotranspiration ETo and water supply) were not used as the present study considers non limiting water conditions. For FAST, each parameter controlling plant source or sink processes in the model were defined by a range of values (Table 1), representative of the diversity found either in the populations studied by Rebolledo *et al.* (2011) or in the literature (e.g., *Tb* and *Kresp*). Sink related parameters considered of minor importance or having small genotypic variability were set to fixed values: first-leaf SLA at 500 $cm^2\ g^{-1}$; and form coefficients for translating blade (length * width) into area at 0.725 and blade length/width at 0.025.

Simulated variables considered for FAST analysis and simulation experiments were as follows:

- plant total and shoot dry weight (g), respectively TDW and SDW
- Total plant non-structural CH_2O concentration (reserve) ($g\ g^{-1}$) (CR)
- Simulated phyllochron (which can be longer that parameter *plasto* under resource limitation) (°Cd)
- Plant total and dead leaf number (respectively, LN and DLN)
- Tiller number per plant (NbT)
- last ligulated leaf blade area on the main stem (cm^2) (LA)

Specifically for the simulation experiment on DR effects on vigour, the median value of ranges defined for each parameter studied in FAST was used, corresponding roughly to the values estimated for a common semi-dwarf rice genotype, IR64 (Luquet *et al.* 2006). Only the parameter *Plasto* was varied and set to 40, 50, 60, 70, 80 and 90 °Cd (corresponding to DR of 0.025, 0.02, 0.017, 0.014, 0.013 and 0.011).

Model behaviour observed in simulation experiments was also compared to the phenotypic data acquired in Exp2 of Rebolledo et al. (2011), on two replications for 190 genotypes for DR and RGR data, and on 50 genotypes for sugar content analyses. On a sub-set of 50 genotypes (randomly chosen to explore the relevance of metabolic phenotyping), sugar content analyses in source leaves (last ligulated leaf on the main stem) were conducted. Non structural carbohydrates were considered to be composed of glucose, fructose, sucrose and starch and were analysed with High Performance Liquid Chromatography (HPLC; see Luquet *et al,* (2006) for details).

RESULTS

Model parameter estimation for the japonica rice panel

Ecomeristem parameters were fitted to the japonica rice panel phenotyped as reported by Rebolledo *et al.* (2011). The distribution of parameter values is shown in Fig. 1. It was normal for all parameters except *Epsib*. *Epsib* (Table 1) exhibited high values probably because it compensated for variation in other source related parameters that were not optimized in this study (*Kdf*, *Kresp*, *Tb*). In Fig. 2 simulated and observed SDW are compared. Model fitting was satisfactory, resulting in an objective function Fo of 9.5% +/- 3.5 across genotypes and considering all fitted parameters. The model tended to slightly underestimate SDW where it was high. Several parameters exhibited significant ($P<0.01$) correlations among each other (Table 2). Such correlations can either be due to physiological or genetic linkages among parameters, considered here as traits. Parameter *MGR* that sets the potential increase of leaf size on successive phytomers was negatively correlated with *Ict*, a physiological threshold parameter for tillering. Consequently, genotypes that show rapid leaf size increase with rank have a low threshold for tiller outgrowth. They also have a long plastochron (*Plasto* parameter) and thus, low DR. In fact, a long constitutive plastochron necessarily limits potential tillering rate because fewer tiller buds are produced. High-*MGR* plants thus develop more slowly and produce fewer tiller buds, but available tiller buds grow out easily.

A positive correlation was observed between *Epsib* (potential radiation use efficiency) and *Ict* (tillering threshold). This would indicate that genotypes converting intercepted light more efficiently

show restrained tiller outgrowth. This does not necessarily mean that they tiller less, because high assimilate supply stimulates tillering. We can provide no evidence here on whether these results have a biological basis or are modelling artefacts.

The observed relative growth rate (RGRobs) on the panel correlated negatively with optimized *Plasto* parameter, and thus positively with DR (reciprocal of plastochron). Parameter *Epsib* had a smaller but also positive effect on RGRobs. Both developmental rate and photosynthetic rate were thus drivers of the vigour observed in terms of RGR.

There was a negative correlation of RGRobs with Fo, the cost function of the optimization process. Low Fo indicates good fit. Consequently, parameter fitting worked better for fast than slow growing plants.

Multiple stepwise, linear regression analysis of optimized model parameter values vs. RGRobs retained *Plasto*, then *Epsib*, then *Ict* and lastly *MGR* (Table 3). The regression model comprising the four model parameters as predictors of RGRobs attributed a negative coefficient to *Plasto* (and thus, a positive one to DR), a positive coefficient to *Epsib*, a negative one to *Ict* and a positive one to *MGR* (which had by far the smallest impact on RGRobs according to the standardized coefficient). Consequently, vigorous genotypes had rapid development (DR), high photosynthetic rates, low tillering threshold (making tillering easier) and, less significantly, large leaves.

Sensitivity analyses

Fig. 3 presents the sensitivity indices of Ecomeristem simulation outputs to 12 of its crop parameters. SDW (Fig. 3a) showed the greatest sensitivity to parameter *Plasto*, both in terms of main and interaction effects. Other parameters that exhibit sensitivity indices higher than 0.1 (*Epsib, Tb, MGR, Ict, SLAp, coeff_R_D1*) were mainly characterized by larger interaction effects compared to main effect, with the exception of parameters *Epsib* and *Tb*.

The output variables LN (Fig. 3c), TN (Fig. 3b) and LA (Fig. 3d) were also strongly influenced by *Plasto*. However =, they were highly sensitive to *MGR* and *Ict*, with larger interaction effects than main effects.

Most parameters thus had little specificity in their effects, due to strong interactions in the simulation process. Effect specificity can be summarized as follows (only total effects > 0.1 considered; **Bold**: at least 50% main effects are involved):

(1) Parameters that set initial plant properties and do not have an impact thereafter:

gdw: TN (0.153) > LN > CR

Lef1: TN (0.207) > LN > CR

Coeff_R_D1: LA (0.233) > CR

These initial parameters had a comparatively small impact on the simulation outcome. Effects were largely interactive and main effects almost absent. The model thus is relatively insensitive to initial values as it simulates numerous compensatory processes constituting phenotypic plasticity.

(2) Parameters that set morphological allometries:

SLAp: LN (0.211) > TN > CR > LA = SDW

LL_BL: LA (0.230) > CR

Observations are similar as for (1).

(3) Parameters that act primarily through assimilate availability:

Epsib: TN (0.159) = LN > **SDW** > CR

Kdf: TN (0.140)

Tb: CR (0.229) > TN > **SDW** = **LN**

Kresp: TN (0.154)

Epsib affects assimilation rate in terms of potential radiation use efficiency (RUE), *Kdf* affects assimilation through light capture, *Tb* affects radiation capture in time through variations of growth duration, and *Kresp* affects the fraction of assimilate available for growth by setting maintenance respiration cost (taxing). These had a surprisingly small impact, partly because only the exponential growth phase was studied here. Before canopy closure, maintenance respiration is small because of small non-photosynthetic biomass, and assimilate gain is more strongly dependent on leaf area growth (driving force of growth exponentiality) than on the photosynthetic rate *per se*. It can thus be expected that these parameters exert greater impact and specificity when studied for the entire growth cycle.

(4) Parameters driving the organogenetic and morphogenetic process:

Plasto: **CR** (0.730) > **SDW** = LA > TN = **LN**

MGR: **LA** (0.496) >> TN > LN > SDW

Ict: TN (0.574) >> LN > LA > CR > SDW

During the exponential growth phase, these parameters had by far the greatest impact on vigour in terms of organ size, number and weight. The parameter *Plasto* which sets the potential organogenetic developmental rate had strong main effects on dry matter accumulation (TDW, SDW) and leaf number (LN) through organ multiplication. Individual leaf area at the end of the simulation was affected in the same sense because potential leaf size is related to leaf position. Opposite effects of similar magnitude (orientation not indicated in Fig. 3) were observed on reserve accumulation (CR) due to trade-offs: The high demand for assimilates under rapid organogenesis left little assimilate surplus for transitory storage.

The main effect of *MGR* was on individual leaf size because this parameter sets potential increase of leaf size from one phytomer to the next. The indirect effects on organ number (TN and LN) had opposite orientation, again because of trade-offs: Producing larger leaves reduces assimilate surplus available for tillering.

Lastly, *Ict* primarily affected tillering, for which it sets the critical level of assimilate availability. This parameter acted almost exclusively through interaction effects because tillering is defined in the model as an adjustment process to assimilate availability.

Relationship between biomass accumulation and developmental rate

The simulations performed for the FAST analysis were used to explore the effect of genotypic DR (reciprocal of parameter *Plasto*) on RGR, across virtual genotypes representing all combinations of parameter values tested in the sensitivity analysis.

All simulation outputs confounded produced a widely scattered but highly significant, positive correlation between RGR and DR (Fig. 4a). The overall trend resembled that observed experimentally on a genetically diverse *Oryza sativa* L. japonica panel as described by Rebolledo *et al.* (2010), reproduced here for comparison (Fig. 4a, black circles).

Simulated effect of DR on growth and sink-source relationships

A simulation experiment was performed to study in more detail the effect of variation of DR on early vigour (Fig. 5). A broad range of *Plasto* parameter values (reciprocal of DR) was forced that represents values occurring in rice. All other model parameters were kept constant at the median value of the ranges provided in Table 1. According to the simulations, high DR (small *Plasto*) was associated with high TDW (Fig. 5a). The corresponding crop growth rates (Fig. 5b) varied accordingly, but showed saturation before the end of simulation for the higher DR levels due to canopy closure (when the simulated LAI exceeds 1.5 and light interception exceeds 75%). The plateau of plant growth rate was associated with a low level of carbohydrate reserves in the plant (Fig. 5c) and onset of senescence of the oldest leaves (Fig. 5e). Strong competition for assimilates associated with rapid DR also caused increased phyllochron as a result of delayed leaf appearance (Fig. 5d).

The accelerated canopy closure under rapid DR was not only due to the greater leaf production on the main stem, but also multiplication of leaf number through greater tillering (Fig. 5f). For the highest levels of DR, tiller number increased early-on due to more rapid production of phytomers that carried tiller buds, but tillering then subsided due to excessive competition. The highest tiller number at the end of simulation was thus simulated for intermediate DR. On the basis of the present parameter settings and the given environment, tillering had a DR optimum between 0.014 and 0.017°Cd^{-1}. The same phenomenon could be observed for plant leaf number (data not presented), with an optimum for DR of 0.02°Cd^{-1}.

Low DR, associated with slow exponential growth, resulted in saturated transitory carbohydrate reserve compartments (assumed to be up to 30% of plant structural biomass in the model) due to limiting sink capacity of growing organs (Fig. 5c; regular oscillations of sugar concentration were due to leaf expansion during a phyllochron). Under these conditions, no leaf senescence occurred. The model prediction that low genotypic DR was associated with increased non-structural carbohydrate pools in the plant was in fact confirmed for the japonica rice panel phenotyped Rebolledo *et al.* (2011). Partial results of this ongoing experimental study are presented in Fig. 4b (on 50 of the 203 genotypes studied).

DISCUSSION

This study provided further evidence for the positive effect of genotypic, organogenetic developmental rate (DR) on early vigour of rice as reported by Rebolledo *et al.* (2011). We used the FSPM Ecomeristem to analyze interactions among physiological traits contributing to early vigour, based on parameter sensitivity analyses and optimization procedures. Ecomeristem, which was developed independently and before the experimental study had been conducted, predicted DR

effects on early plant dry matter accumulation much in the way it was observed experimentally. In particular, the simulated trend of DR effects on RGR across a population of diverse virtual genotypes was similar compared to the observations on a genetically diverse panel, phenotyped for early vigour and its components.

Model sensitivity analysis: strong effects of organogenetic parameters on vigour

According to the FAST sensitivity analysis, model parameters governing structural development (*Plasto*, *MGR*, *Ict*) had far greater effects on early vigour than parameters setting initial morphology at germination, leaf/sheath or shoot/root allometries, or rates of resource acquisition and conversion. This stands in contrast to the behaviour of conventional crop models that drive assimilate partitioning in a more prescriptive and less interactive way, resulting in greater model sensitivity to supply related parameters, as opposed to structural demand (unpublished data by the authors; Fourcaud *et al.* 2008).

Plasto had a particularly strong effect on vigour, for several reasons. It drives directly the rate of leaf number production on a given culm, indirectly the rate of branching through the frequency of tillering cycles related to phytomer development, and also indirectly the rapidity of leaf expansion because in rice, leaf blade expansion is chiefly accomplished within a single phyllochron period (Luquet *et al.* 2006). In other grasses, the orchestration of expansion of successive leaves is frequently different. In maize and sorghum, several leaves expand simultaneously (Andrieu *et al.* (2006); Chenu *et al.* (2007) for maize; Kouressy *et al.* (2008) for sorghum).

Organogenetic DR sets the rate of sink generation in the plant, with some of the sinks (leaves) turning into sources thereafter. DR is thus an intrinsic pacemaker of plant development, i.e. the construction of its body plan (Miyamoto *et al.* 2004), which is comparatively rigid in grasses. Whether or not DR has a positive effect on early vigour (SDW, RGR) in a given environment would thus depend on the extent to which the plant can balance its C source-sink relations through the regulation of other sinks such as tillering or leaf size: the greater the plasticity of the organogenetic and morphogenetic process, the more DR effects would be compensated by adjustment processes such as tillering and organ size variation, and the less genotypic DR would impact on early vigour.

Since the complexity of the adjustment processes and their underlying behavioural rules is difficult to study experimentally, Ecomeristem's prediction of a decisive influence of DR on early vigour needs to be interpreted with caution. Two pieces of evidence, however, supported it: (1) observed effects of DR on vigour across a great diversity of japonica rice genotypes (Rebolledo *et al.* 2011) were of similar magnitude as those predicted by the model for a similarly broad range of virtual genotypes

(Fig. 4); and (2) in the same diversity panel, low-DR and low-vigour genotypes tended to accumulate more transitory reserves, a trend that the model predicted (Fig. 4, Fig. 5). Also, the contribution of observed genotypic variation of DR to RGR for the phenotyped rice panel (Rebolledo *et al.* 2011) was similar to the contribution of optimized *Plasto* parameter values to RGR in the present study (Tables 2 & 3).

Organogenetic parameters *Ict* and *MGR* were found to have a lesser and more specific effect than *Plasto* on the simulation outcome. *Ict* mainly acted on tiller number and *MGR* on leaf size in accordance with their physiological function, although both exerted strong interactive effects on other processes and interact particularly strongly with *Plasto*, which remained the most influential parameter on early vigour.

The strong positive association of DR with tiller production is supported by several studies (Morita *et al.* 2005; Rebetzke and Richards 1999; W. ter Steege *et al.* 2005). Evidence for a physiological linkage of DR and tillering was found in mutants. For example, in rice mutants having modified plastochron (Asai *et al.* 2002; Itoh *et al.* 1998; Miyoshi *et al.* 2004) or tillering (Komatsu *et al.* 2003; Zou *et al.* 2005), the physiological linkage between tillering and DR was not overcome and the phenotype of the mutants was systematically modified on both traits as compared to the wild type. Similar evidence on physiological trait linkages (based on the genetic modification of a single gene) was also shown for leaf size vs. DR (Itoh *et al.* 1998; Miyoshi *et al.* 2004). Rice mutants affected on a gene controlling the rate of leaf primordium initiation had modified leaf size. While there is strong evidence for physiological linkages and trade-offs among tillering, DR and/or leaf size (e.g. for wheat, W. ter Steege *et al.* (2005) and rice, Miyamoto *et al.* (2004)) some recent studies suggested that they also have a genetic basis: Tisné *et al.* (2008) suggested a genetic linkage between leaf cell number and size with plant leaf number in *Arabidopsis thaliana* L, and W. ter Steege *et al.* (2005) reported for wheat genetic linkages among tiller number, leaf size and leaf number. This is consistent with the results provided by Ecomeristem in this study, showing correlations among complementary genotypic parameters observed on the japonica panel. However, the simulation experiment also indicated strong physiological interactions among the same traits on the phenotype.

Comparatively small effects on vigour of other structural parameters

The parameter SLAp showed small and interactive effects on plant vigour (in terms of SDW, Fig. 5). This contrasts with studies of plant functional ecology proposing a central role for SLA in plant vigour (Osone *et al.* 2008; Poorter 1999; Rebetzke *et al.* 2004; Shipley 2006). High SLA (small leaf dry weight per leaf area) reduces photosynthetic capacity but enables producing a greater leaf area per unit

assimilate invested, thus accelerating canopy closure. This was observed experimentally for upland rice (Dingkuhn *et al.* 1999) but Asseng *et al.* (2003) demonstrated in a model-assisted study that beneficial effects of increased SLA are small in wheat. Measurements of SLA generally include structural and non-structural dry matter, whereas the *SLAp* parameter of Ecomeristem only acts on the structural component (the simulated SLA then including both structural and non-structural (reserve) components). It is possible that the association of low SLA with poor early vigour in rice is in part due to higher leaf reserve status, in turn caused by structural sink limitation, as indicated by the present results.

Initial parameters, i.e. *gdw*, *lef1* and *coeff_R_D1* (respectively seed DW, length of the first leaf, initial root-shoot wt ratio) showed only small effects on plant vigour. This was mainly caused by the trait interactions and compensation processes simulated in the course of development, rendering the outcome less dependent on initial conditions. By contrast, Rebetzke *et al.* (2004), Rebetzke and Richards (1999) and Rebetzke *et al.* (2007) proposed that dimensions of the 1st or 2nd leaf are predictive of early vigour. In Ecomeristem, leaf size effects on vigour are chiefly translated through *MGR* (the potential size increase rate for successively appearing leaves). It remains to be studied to what extent large size of early leaves translates into rapid size increase during ontogenesis, parameters that may be physiologically linked.

Source related parameters

Surprisingly, the sensitivity indices computed for parameters involved in carbon acquisition (*Kdf*, *Kresp*, *Epsib*) were generally much smaller and more interactive than those of sink related parameters. Before canopy closure, maintenance respiration is small because of small non-photosynthetic biomass, and assimilate gain is more strongly dependent on leaf area growth than on the photosynthetic rate *per se*. This is also true for base temperature (*Tb*) which, in a given thermal environment, sets the thermal time elapsing per day, and thus the amount of radiation that can be harvested per phyllochron or per growth phase. However, despite the small effects of *Epsib* in the model sensitivity analysis, this parameter experimentally came out second after DR as a predictor of RGRobs (multiple linear regression analysis, Table 3). A strong physiological synergy could be expected between photosynthetic potential (source) and DR (sink). In fact, C4 grasses with high photosynthetic rates such as sorghum (Clerget *et al*. 2008) and maize (Birch *et al*. 1998) have higher DR than C3 grasses such as rice (Rebolledo *et al.* 2011).

Developmental rate vs. dynamics of sink-source relationships

Simulations indicated that high genotypic DR had a strong positive impact on plant growth rate during the exponential growth phase. However, it accelerated canopy closure and thus inter- and intra-plant competition for light and thus, C assimilates. Consequently, crop growth rate levelled off earlier (Fig. 5).

Internal competition for resources may also feedback on DR. Ecomeristem predicted an increase of phyllochron through assimilate shortage as high DR and vigour led to an early end of exponential growth (Fig. 5d). This prediction cannot be validated here but it is known that competition for resources, for example through close spacing (Ouknider *et al.* 1991) or a reduction in incident light intensity (Lafarge *et al.* 2010) increases phyllochron. Birch et al. (1998) reported acclimation effects of temperature and light on phyllochron in maize.

Consequences for further improvement of crop models

If the present findings, and those of the companion paper (Rebolledo *et al.* 2011), can be validated for different environmental conditions and at agronomic scale, they are of significance for crop improvement. The results also make a case for the improvement of crop models, not only for the purpose of predicting early vigour, but more generally to capture important physiological and genetic determinants of crop performance that current crop models mostly do not consider. This does not necessarily require architecturally detailed FSPMs (most of which have only little physiological bases), even if considering plant architecture enables to account for the (geometric, topologic) distance between organs and thus better to simulate transport and signalling processes. But it calls for the simulation of interactions between development processes (determining structure and sinks) and resource acquisition (source processes) in general (Chenu *et al.* 2009; Hammer *et al.* 2010). This, in turn, requires simulation of feedbacks between source and sink processes which are located in different organs and therefore are based on signalling in a broad sense. In Ecomeristem, a generic signal is simulated in the form of Ic (aggregate supply / aggregate demand at the plant scale). This state variable can be interpreted as a signal of assimilate availability and we use it as a proxy for sugar signalling (Rognoni *et al.* 2007; Rolland *et al.* 2006; Stitt *et al.* 2007). Organ or process specificity of signalling is thereby located on the receptor side, simulated as response law of the organ concerned.

It may eventually be necessary to define different signals for different types of physiological feedbacks, and to simulate signal transduction processes in order to capture proximity effects. On the other hand, even Ecomeristem with its comparatively small number of parameters showed extremely complex behaviour due to the process interactions brought about by a single, generic

signal state variable. Proceeding further along the concepts of this model is necessary to capture essential system properties, but must be pursued with caution to ensure appropriate parameterization and experimental validation.

Conclusion

This study used the FSPM Ecomeristem to understand experimental evidence on the effects of genotypic DR on early vigour, observed on a diverse panel of japonica rice germplasm under stress free conditions as reported in the companion paper. Sensitivity analysis confirmed *Plasto* (1/ DR) as the most influential parameter for early growth vigour during the exponential growth phase. Simulation of a broad range of virtual genotypes gave growth vs. DR trends comparable to observations on the japonica rice panel. The authors conclude that crop models, if they are to be used broadly for the evaluation of varietal trait combinations in the context of crop improvement objectives, need to accurately simulate feedbacks between developmental (organogenetic) processes and resources acquisition and conversion processes.

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TABLES

Table 3 : list of Ecomeristem parameters considered in the sensitivity analysis performed with FAST method and corresponding range of values.

Parameter name and definition	range
Initial parameters	
gdw: initial parameter, grain dry weight (g)	0.015 to 0.03
Lef1: initial parameter, final length (blade+sheath) of the first leaf presenting differentiated blade and sheath (cm)	5 to 8
coeff_R_D1: initial parameter, initial root/shoot dry weight ratio (unitless)	0.8 to 1
Allometric parameters	
SLAp: slope parameter of the negative logarithmic equation computing SLA for successive leaf ranks ($cm^2\ g^{-1}$)	30 to 80
LL_BL: ratio between leaf and blade length ($cm\ cm^{-1}$)	1.3 to 1.8
Source acquisition parameters	
Epsib: radiation conversion efficiency into biomass (shoot, root, storage, maintenance respiration) ($g\ MJ^{-1}\ m^{-2}$)	3 to 6.5
Kdf: light extinction coefficient by crop (unitless)	0.5 to 0.7
Tb: base temperature (°C)*	9 to 14
Kresp: coefficient of maintenance respiration (Q_{10}=2 law)	0.01 to 0.025
Morphogenetic parameters	
Plasto: plastochron (°Cd)	40 to 90
MGR: Meristem Growth Rate (cm)	5 to 13
Ict: Ic threshold value for tillering (unitless)	0.5 to 2.5

* In the context of this study, Tb affects phase duration and thus, light resources per unit thermal time.

Table 2. Correlation matrix among optimized Ecomeristem crop parameters *MGR*, *Ict*, *Epsib* and *Plasto* (definition in Table 1), the value of the cost function of optimization (Fo) and the observed relative crop growth rate (RGRobs), based on phenotyping of a 190 japonica rice panel as reported by Rebolledo *et al.* (2011).

Variables	*MGR*	*Ict*	Epsib	*Plasto*	Fo	RGRobs
Ict	**-0.402**	**1**				
Epsib	0.001	**0.528**	**1**			
Plasto	**0.468**	**-0.227**	0.190	**1**		
Fo	-0.088	0.130	0.052	0.205	**1**	
RGRobs	0.097	-0.108	**0.243**	**-0.411**	**-0.331**	**1**

Values in bold print are different from 0 (P<0.01)

Table 3. Stepwise multiple linear regression analysis explaining observed relative growth rate (RGRobs) with the Ecomeristem crop parameters (Table 1) optimized to fit phenotyping results on a 190 japonica rice panel (Rebolledo *et al.* 2011).

Predictor variables and R^2 of model

	MGR	*Ict*	*Epsib*	*Plasto*	Adjusted R^2
1 variable				X	0.17
2 variables			X	X	0.28
3 variables		X	X	X	0.52
4 variables	X	X	X	X	0;55

Model coefficients

	Coef	Stdev	Std Coef	t-ratio	P
Constant	1.018	0.000595	0.000	1709.;68	$<10^{E}$-20
MGR	0.00011	0.000032	0.235	3.32	0.00058
Ict	-0.00120	0.000180	-0.540	-6.78	$<10^{E}$-20
Epsib	0.00056	0.000061	0.682	9.09	$<10^{E}$-20
Plasto	-0.00011	0.000009	-0.786	-11.23	$<10^{E}$-20

Analysis of variance

	DF	SS	MS	F	P
Regression	4	0.000051	<10 E-8	1.80E+308	$<10^{E}$-20
error					
Residuals	127	0.000039	3.1 10^{E}-7		
Total	131	0.000091			

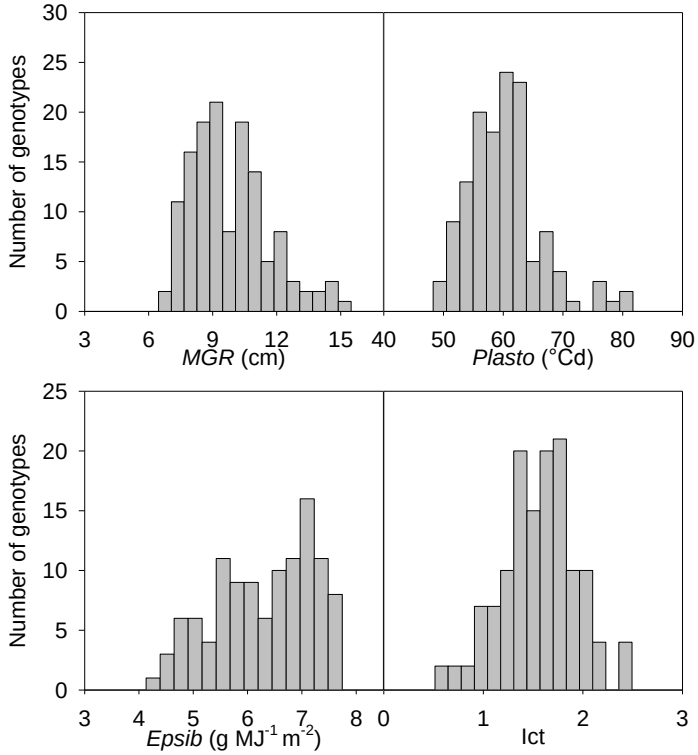

Figure 1 *: Distribution of parameter values optimized on the panel of 190 japonica rice genotypes studied in Exp2 of Rebolledo et al. (2011).*

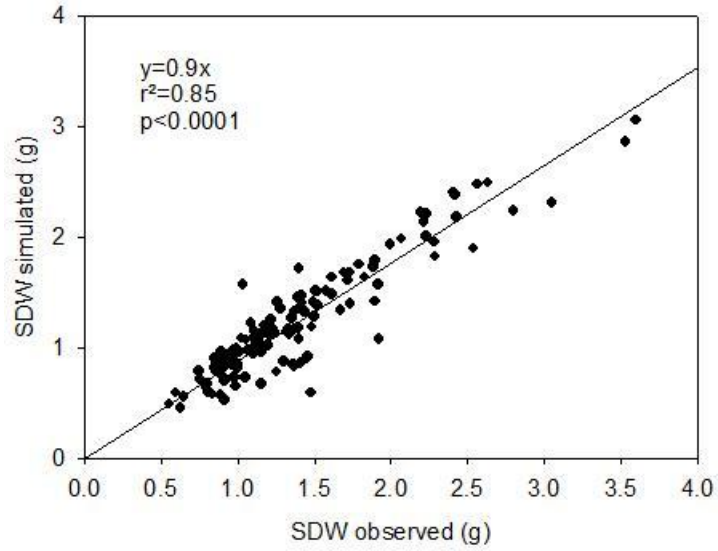

Figure 2 : *Comparison between average simulated and observed shoot dry weight (SDW) of the experiment Exp2 described in Rebolledo et al. (2011) on a panel of 190 japonica rice genotypes; simulated values were obtained for each genotype using the parameters optimized on Exp2 observations***.**

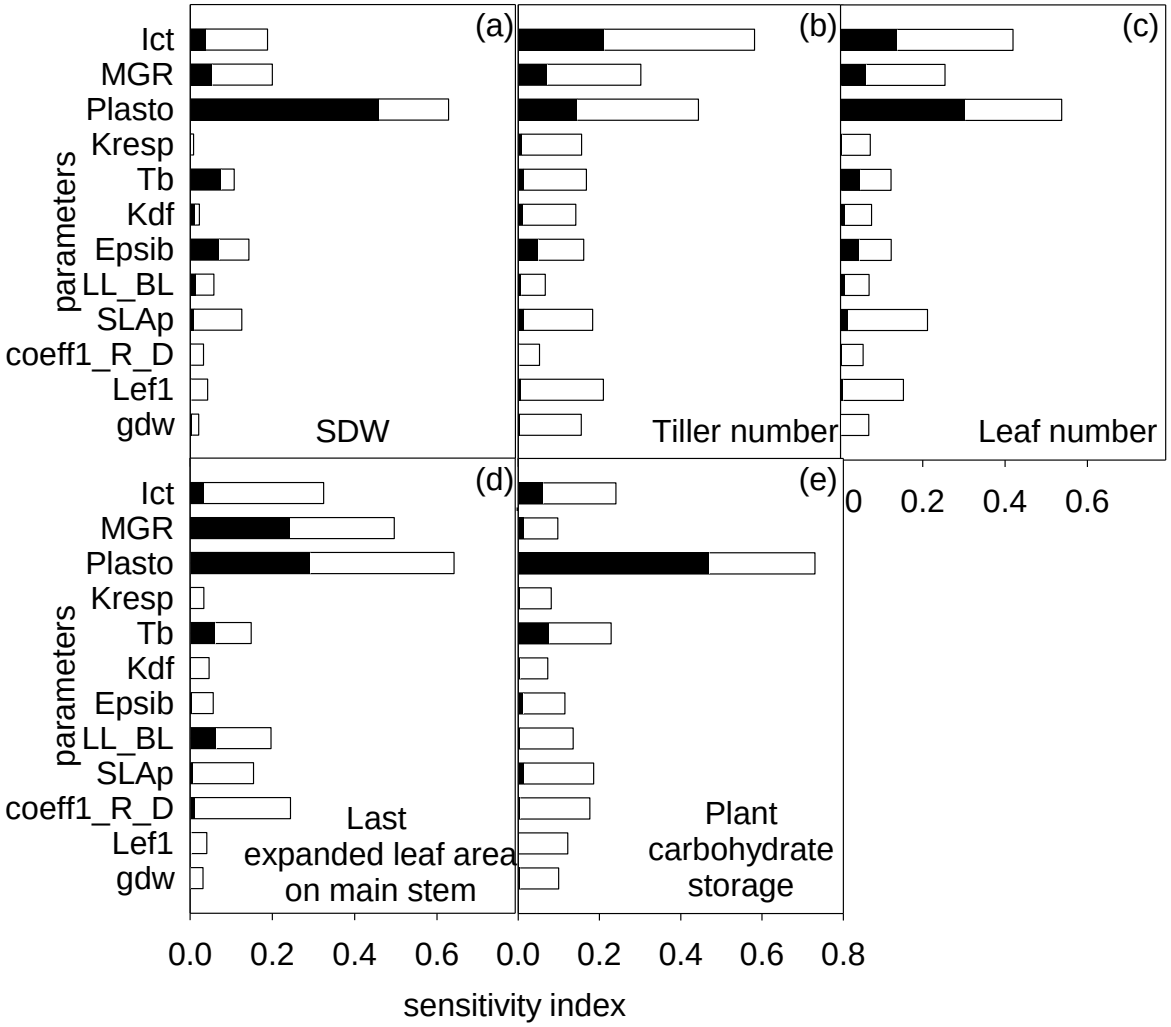

Figure 3 *: Sensitivity analysis results using Fourier Amplitude Sensitivity Test method (FAST) and describing Ecomeristem model sensitivity to 15 of its parameters. Parameters are listed in Table 1. Sensitivity index were calculated for simulated (a) plant shoot (SDW, g), (b, c) tiller and leaf numbers, (d) last ligulated leaf area on the main stem (cm²) and (e) plant carbohydrate reserve (in g of CH_2O per g of shoot). Sensitivity indices are additive (white bar: main effect, black bar: interaction effect).*

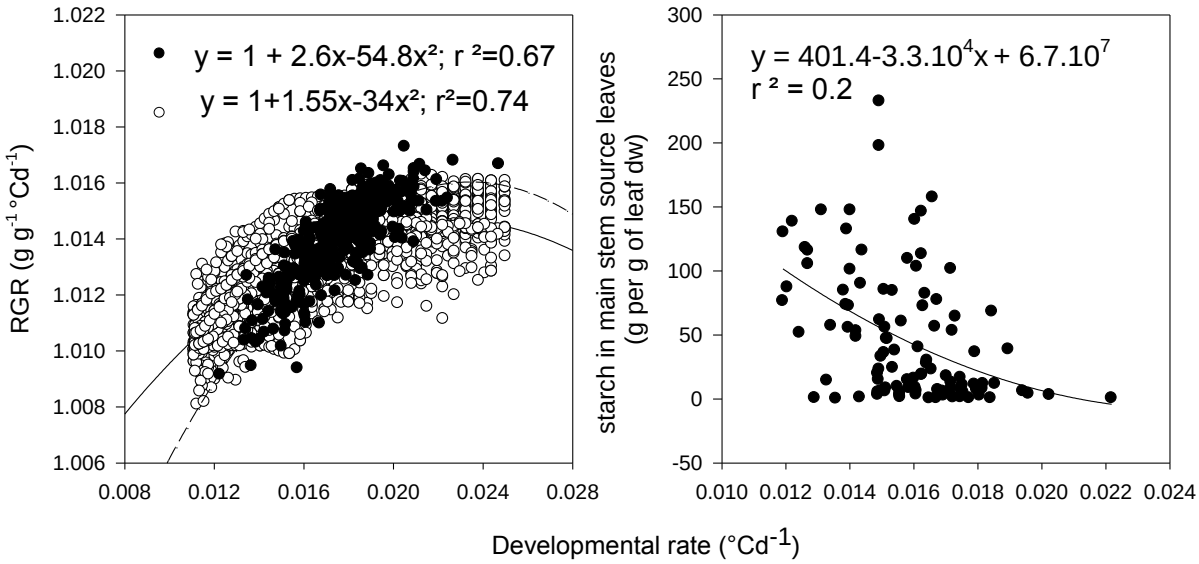

***Figure 4**: Relationship between developmental rate (inverse of phyllochron, °Cd^{-1}) and (left): Relative Growth Rate (RGR, g g^{-1} °Cd^{-1}) and (right): starch content in the youngest source leaf on main stem. White circles: across simulations performed in the sensitivity analysis presented in Figure 2; black circles: for individual plants in replication 1 and 2 of Experiment Exp2 presented in Figure 3 of Rebolledo et al. (2011) (Left: 200 genotypes; Right: 50 genotypes). Plain curve: regression corresponding to black symbols: Dashed curve: regression corresponding to white symbols (see equations in the figure).*

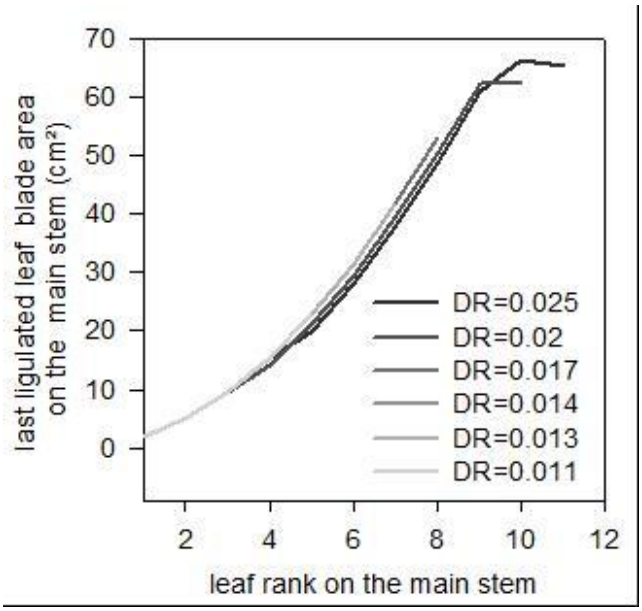

***Figure 6:** simulation of final leaf blade area on the main stem as a function of rank for 6 values of developmental rate (DR, °C.d^{-1}, reciprocal of* Plasto *parameter in the model).*

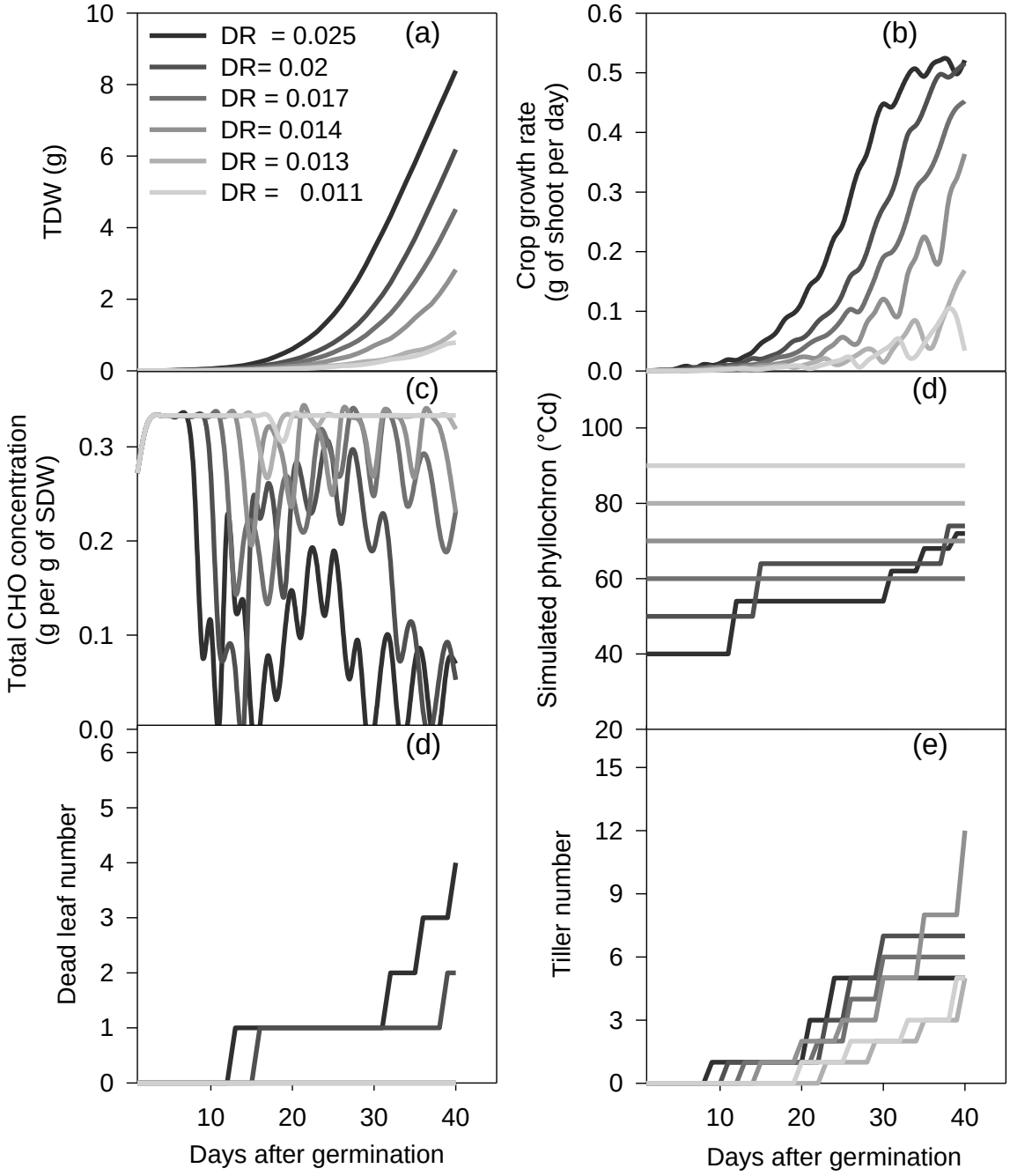

Figure 5 *: dynamic simulation of plant total dry weight (TDW, a) and growth rate (b), carbohydrate concentration (CH_2O, c), simulated phyllochron (d), dead leaf number (e) and tiller number (f) for 6 values of developmental rate (DR, in °Cd^{-1}, inverse of* Plasto *parameter in the model).*

Appendix II : Varietal table

Detailed table for genotypes studied in this PhD thesis. The table associates to each genotype a reference ATPd number (Orytage number), Germplasm name , Nucleus (Orytage Pelgreen Nucleus), Country (Seeds origin), Varietal Group (Tjap= Tropical Japonica, TempJap=Temperate Japonica, Indica, Aus, Aromatic), Ecosystem of adaptation (U=Upland, RL=Rainfed Lowland, I=Irrigated), Breeding type (T=Traditional, I=Improved), Varieties studied in article(Chapter) 1, 2 and 3 are pointed with “1”; The number of the cluster for chapters 2 and 3 are also detailed.

ATPd	Germplasm name	Nucleus	Country	Varietal group	Ecosystem of adptation	Breeding Type	Article 1	Article 2	Cluster Article 2	Article 3	Cluster Article 3
4	AZUCENA	1	PHILIPPINES	tjap	U	T	1	1	2	1	1
13	GIZA 171	1	EGYPT	tempjap	I	I	1			1	3
14	IAC 165	1	BRAZIL	tjap	U	I	1	1	2	1	2
15	IR64	1	PHILIPPINES	indica	I	I	1	1	1	1	3
21	MOROBEREKAN	1	GUINEA	tjap	U	T	1	1	2	1	1
23	NIPPONBARE	1	JAPAN	tempjap	I	I	1			1	2
283	COLOMBIA 1		COLOMBIA	tjap	U	I	1			1	2
319	IAC 25		BRAZIL	tjap	U	I	1	1	2		
338	IR72967-12-2-3		PHILIPPINES	indica	I	I	1	1	3	1	3
339	IRAT 104		IVORY COAST	tjap	U	I					
342	IRAT 13		IVORY COAST	tjap	U	I					
369	KETAN MENAH		INDONESIA	tjap	U	T	1	1	2		
372	KINDANG PATONG		PHILIPPINES	tjap	U	T	1	1	3	1	1
399	OS 6		ZAIRE	tjap	U	T	1	1	2	1	1
999	EARLY MUTANT IAC 165		BRAZIL	tjap	U	I	1	1	3	1	1
19	M 202	1	USA	tempjap	I	I	1	1	2	1	3
22	N 22	1	INDIA	aus	U	T	1	1	1	1	1
25	TEQUING	1	CHINA	indica	I	I	1	1	1	1	3
250	62667		SENEGAL	tjap	U	T	1			1	3
251	63-104		SENEGAL	tjap	U	T	1			1	1
252	ARAGUAIA		BRAZIL	tjap	U	I	1			1	1
253	ARIAS		INDONESIA	tjap	U	T	1			1	1
254	ARROZ CEBADA		VENEZUELA	tjap	U	T	1			1	2
255	BABER		INDIA	tjap	U	T	1			1	2
256	BAGAN ASALAO		MALAYSIA	tjap	U	T	1			1	1
257	BAKUNG H		MALAYSIA	tjap	U	T	1			1	1
258	BELOHAKILA 119		MADAGASCAR	indica	NA	T	1			1	3
259	BENGALY VAKARINA		MADAGASCAR	tjap	U	T	1			1	1
260	BICO BRANCO		BRAZIL	tjap	U	T	1	1	1	1	2
261	BINULAWAN		PHILIPPINES	tjap	U	T	1			1	1
262	BODA 148-3		MADAGASCAR	tjap	U	T	1			1	1
263	BOTRA FOTSY		MADAGASCAR	tjap	U	T	1			1	1
265	CAAWA/FORTUNA 6		PHILIPPINES	tjap	U	I	1			1	1
266	CAIAPO		BRAZIL	tjap	U	I	1			1	1
267	CANA ROXA		BRAZIL	tjap	U	T	1			1	1
268	CANELA DE FERRO		BRAZIL	tjap	U	T	1			1	1
269	CHA LOY OE		THAILAND	tjap	U	T	1			1	3
270	CHA PHU MA		THAILAND	tjap	U	T	1	1	2	1	1
273	CICIH BETON		INDONESIA	tjap	NA	T	1			1	2
274	CIRAD 141		BRAZIL	tjap	U	I	1				
275	CIRAD 358		IVORY COAST	tjap	U	I	1				
276	CIRAD 392		MADAGASCAR	tjap	U	I	1			1	2
277	CIRAD 394		MADAGASCAR	tjap	U	I	1	1	1	1	2
278	CIRAD 402		BRAZIL	indica	U	I				1	2
279	CIRAD 403		Unknown	tjap	U	I	1			1	2

ATPd	Germplasm name	Nucleus	Country	Varietal group	Ecosystem of adptation	Breeding Type	Article 1	Article 2	Cluster Article 2	Article 3	Cluster Article 3
280	CIRAD 409		Unknown	tjap	U	I	1			1	2
281	CIRAD 488		MADAGASCAR	tjap	U	I	1	1	1	1	1
282	CNA-7\BO\1\1>33-13-6-1		COLOMBIA	tjap	U	I	1			1	2
1	APO	1	PHILIPPINES	indica	U	I	1	1	1	1	3
284	CT13582-15-5-M		COLOMBIA	tjap	U	I	1	1	3	1	2
289	CUBA 65		CUBA	tjap	U	T	1	1	2	1	1
290	CUIABANA		BRAZIL	tjap	U	I				1	1
291	CURINCA		BRAZIL	tjap	U	I	1			1	3
292	DAM		THAILAND	tjap	U	T	1			1	1
293	DANGREY		BHUTAN	tjap	NA	T					
294	DAVAO		PHILIPPINES	tjap	U	T	1			1	1
295	DAWASAN RED		BHUTAN	tjap	NA	T					
296	DINORADO		PHILIPPINES	tjap	U	T	1			1	1
297	DOURADO AGULHA		BRAZIL	tjap	U	T	1	1	2	1	1
298	DOURADO PRECOCE		BRAZIL	tjap	U	T	1	1	2		
300	ESPERANZA		BOLIVIA	tjap	U	T	1			1	2
301	FOHISOMOTRA		MADAGASCAR	tjap	U	T	1				
302	FOSSA HV		BURKINA FASSO	tjap	U	T	1	1	1	1	3
303	GANIGI		INDONESIA	tjap	U	T	1			1	1
304	GEMJYA JYAM		BHUTAN	tjap	NA	T	1			1	3
305	GOGO		INDONESIA	tjap	U	T	1			1	1
307	GOGO LEMPAK		INDONESIA	tjap	U	T	1			1	1
306	GOGO LEMPUK		INDONESIA	tjap	U	T	1			1	1
308	GOMPA 2		INDIA	tjap	U	T	1			1	3
309	GOTAK GATIK		INDONESIA	indica	NA	T	1	1	1		
310	GRAZI		IVORY COAST	tjap	U	T	1			1	1
311	GUARANI		BRAZIL	tjap	U	I	1			1	1
312	GUNDIL KUNING		INDONESIA	tjap	U	T	1				
313	HAIFUGOYA		TAIWAN	tjap	U	T	1			1	2
314	HAWM OM		THAILAND	tjap	U	T	1				
315	HD 1-4		FRANCE	tjap	U	I	1			1	1
2	ASD 1	1	INDIA	indica	I	T	1	1	1	1	3
320	IAC 47		BRAZIL	tjap	U	I	1			1	1
321	IDSA 77		IVORY COAST	tjap	U	I	1			1	2
322	IGUAPE CATETO		BRAZIL	tjap	U	T	1			1	3
323	INDANE		MYANMAR	tjap	U	T	1			1	1
458	IR47684-05-1-B		PHILIPPINES	tjap	U	I	1			1	2
446	IR47686-09-01-B-1		PHILIPPINES	tjap	U	I	1			1	1
447	IR53236-275-1		PHILIPPINES	tjap	U	I	1			1	1
325	IR60080-46A		PHILIPPINES	tjap	U	I	1			1	3
326	IR63371-38		PHILIPPINES	tjap	U	I	1			1	2
328	IR63372-08		PHILIPPINES	tjap	U	I	1			1	2
327	IR63380-08		PHILIPPINES	tjap	U	I	1			1	2
449	IR65261-19-1-B		PHILIPPINES	tjap	U	I	1			1	1
330	IR65907-116-1-B		PHILIPPINES	tjap	U	I	1			1	2

ATPd	Germplasm name	Nucleus	Country	Varietal group	Ecosystem of adptation	Breeding Type	Article 1	Article 2	Cluster Article 2	Article 3	Cluster Article 3
451	IR65907-173-1-B		PHILIPPINES	tjap	U	I	1			1	2
331	IR65907-188-1-B		PHILIPPINES	tjap	U	I	1			1	3
452	IR65907-206-4-B		PHILIPPINES	tjap	U	I	1			1	2
332	IR66421-096-2-1-1		PHILIPPINES	tjap	U	I	1			1	3
453	IR66421-105-1-1		PHILIPPINES	tjap	U	I	1			1	2
334	IR68704-145-1-1-B		PHILIPPINES	tjap	U	I	1			1	2
335	IR70758-17-2-1		PHILIPPINES	indica	U	I	1			1	3
454	IR71524-44-1-1		PHILIPPINES	tjap	U	I	1			1	2
336	IR71525-19-1-1		PHILIPPINES	tjap	U	I	1	1	3	1	2
337	IR71676-90-2-2		PHILIPPINES	indica	I	I	1			1	3
6	BASMATI 370	1	INDIA	aro	I	T	1	1	1	1	3
7	BULU PANDAK	1	INDONESIA	tjap	RL	T	1	1	3	1	3
340	IRAT 109		IVORY COAST	tjap	U	I	1			1	2
341	IRAT 112		IVORY COAST	tjap	U	I	1			1	3
8	DOM SOFID	1	IRAN	aro	I	T	1	1	3	1	3
343	IRAT 144		GHANA	tjap	U	I	1			1	2
344	IRAT 170		IVORY COAST	tjap	U	I	1			1	3
345	IRAT 177		FRENCH GUYANA	tjap	U	I	1			1	3
346	IRAT 2		SENEGAL	tjap	U	T	1				
347	IRAT 212		IVORY COAST	tjap	U	I	1			1	3
348	IRAT 216		IVORY COAST	tjap	U	I	1			1	2
349	IRAT 234		GUF-BRA	tjap	U	I	1			1	1
350	IRAT 257		BRAZIL	tjap	U	I	1			1	2
351	IRAT 335		BOLIVIA	tjap	U	I					
352	IRAT 362		NICARAGUA	tjap	U	I	1			1	2
353	IRAT 364		NICARAGUA	tjap	U	I	1			1	1
354	IRAT 366		NICARAGUA	tjap	U	I	1			1	3
355	IRAT 380		MADAGASCAR	tjap	U	I	1			1	2
356	JAO HAW		THAILAND	tjap	U	T	1	1	3	1	2
357	JIMBRUK JOLOWORO		INDONESIA	tjap	U	T	1			1	1
358	JUMALI		NEPAL	tjap	U	T	1			1	3
359	JUMULA 2		NEPAL	tjap	U	T	1			1	3
360	KAKANI 2		NEPAL	tjap	U	T	1			1	3
361	KANIRANGA		INDONESIA	tjap	U	T	1			1	1
362	KARASUKARA SURANKASU		TAIWAN	tjap	U	NA	1			1	1
363	KEDAYAN		MALAYSIA	tjap	U	T	1			1	1
365	KENDINGA 5 H		MALAYSIA	tjap	U	T	1	1	3	1	1
366	KERITING TINGGI		Unknown	indica	NA	T	1			1	3
367	KETAN KONIR		INDONESIA	tjap	RL	T	1				
368	KETAN LUMBU		INDONESIA	tjap	U	T	1			1	3
9	DULAR	1	INDIA	aus	U	T	1			1	2
370	KETAN SELEH		INDONESIA	indica	RL	T	1			1	3
371	KHAO KAP XANG		LAOS	tjap	U	T	1			1	1
10	FANDRAPOTSY 104	1	MADAGASCAR	indica	I	T	1	1	2	1	2
373	KOMOJAMANITRA		MADAGASCAR	tjap	U	T	1			1	1

ATPd	Germplasm name	Nucleus	Country	Varietal group	Ecosystem of adptation	Breeding Type	Article 1	Article 2	Cluster Article 2	Article 3	Cluster Article 3
374	KU 115		THAILAND	tjap	U	I	1			1	1
375	KUROKA		JAPAN	tempjap	I	I	1			1	3
376	LAC 23		LIBERIA	indica	U	I	1			1	3
377	LAMBAYQUE 1		PERU	tjap	U	T	1			1	3
378	LOHAMBITRO 3670		MADAGASCAR	indica	U	T	1			1	2
379	LUDAN		PHILIPPINES	tjap	U	T	1			1	1
380	MA HAE		THAILAND	tjap	U	T	1			1	3
381	MAINTIMOLOTSY 1226		MADAGASCAR	tjap	U	T	1	1	3	1	3
382	MALAGKIT PIRURUTONG		PHILIPPINES	tjap	U	T	1			1	1
383	MANELATRA 520		MADAGASCAR	tjap	U	T	1			1	3
384	MANDRIRAVINA 3512		MADAGASCAR	tjap	U	T					
385	MARAVILHA		BRAZIL	tjap	U	I	1			1	2
386	MED NOI		THAILAND	indica	NA	T	1			1	1
387	MITSANGAHIJERY		MADAGASCAR	tjap	U	T	1			1	1
388	MOLOK		INDONESIA	tjap	RL	T	1			1	3
389	NABESHI		TAIWAN	tjap	NA	NA	1			1	3
390	NEP HOA VANG		VIETNAM	tjap	U	T	1			1	3
391	NHTA 10		INDIA	tjap	U	T	1			1	1
392	NHTA 5		INDIA	tjap	U	T	1			1	1
393	NPE 253		PAKISTAN	tjap	U	T				1	3
395	NPE 826		PAKISTAN	tjap	U	T					
397	ORYZICA SABANA 6		COLOMBIA	tjap	U	I	1			1	3
398	OS 4		ZAIRE	tjap	U	T	1			1	1
17	KHAO DAM	1	THAILAND	tjap	U	T	1	1	2	1	1
400	P5589-1-1-3-P		COLOMBIA	tjap	U	I	1			1	2
401	PACHOLINHA		BRAZIL	tjap	U	T	1			1	2
403	PADI BOENAR		INDONESIA	tjap	RL	T	1	1	3	1	2
404	PADI KASALLE		INDONESIA	tjap	NA	T	1			1	3
406	PADI ROTAN		INDONESIA	tjap	RL	T	1	1	3	1	3
407	PALAWAN		PHILIPPINES	tjap	U	T	1			1	2
409	PATE BLANC MAN 1		IVORY COAST	tjap	U	T	1			1	1
410	PCT11\0\0\2,BO\1>55-1-3-1		COLOMBIA	tjap	U	I	1			1	2
412	PCT4\SA\4\1>1076-2-4-1-5		COLOMBIA	tjap	U	I	1			1	2
413	PEH PI NUO		CHINA	tjap	NA	T	1	1	2	1	1
414	POENOET HITAM		INDONESIA	tjap	U	T	1			1	2
415	PRATAO		BRAZIL	indica	na	T	1	1	1		
459	PRIMAVERA		BRAZIL	tjap	U	I	1	1	1	1	2
416	PULU LAPA		INDONESIA	tjap	U	T					
417	RATHAL		SRI LANKA	tjap	U	T	1			1	1
418	REKET MAUN		INDONESIA	tjap	RL	T	1	1	3	1	2
419	RT 1031-69		ZAIRE	tjap	U	T	1			1	1
420	SA TANG		LAOS	tjap	U	T	1				
421	SEBOTA 65		BRAZIL	indica	U	I	1			1	3
422	SENG		THAILAND	tjap	U	T	1			1	1
424	SPEAKER		PHILIPPINES	tjap	U	T	1			1	1

ATPd	Germplasm name	Nucleus	Country	Varietal group	Ecosystem of adptation	Breeding Type	Article 1	Article 2	Cluster Article 2	Article 3	Cluster Article 3
427	TANDUI		MALAYSIA	tjap	U	T	1			1	2
428	TREMBESE		INDONESIA	tjap	U	T	1			1	1
429	TRES MESES		BRAZIL	tjap	U	T	1			1	1
430	TSIPALA 89		MADAGASCAR	tjap	U	T	1			1	1
432	VARY LAVA 90		MADAGASCAR	tjap	U	T	1			1	1
433	VARY LAVA DE BETAFO		MADAGASCAR	tjap	U	T	1				
434	VARY MADINIKA 3566		MADAGASCAR	tjap	U	T	1			1	1
435	VARY MALADY		MADAGASCAR	tjap	U	T	1			1	1
436	VARY MANANELATRA		MADAGASCAR	tjap	U	T					
437	VARY SOMOTRA SIHANAKA		MADAGASCAR	tjap	U	T	1			1	3
455	VIETNAM1		VIETNAM	tjap	U	T	1			1	1
456	VIETNAM2		VIETNAM	tjap	U	T	1	1	1	1	2
457	VIETNAM3		VIETNAM	tjap	U	T	1	1	3	1	2
438	WAB 56-125		IVORY COAST	tjap	U	I	1			1	2
439	WAB 56-50		IVORY COAST	tjap	U	I	1			1	2
440	WAB706-3-4-K-KB-1		IVORY COAST	tjap	U	I	1			1	1
442	Y CHANG JU		TAIWAN	tempjap	NA	T	1				
443	YANCAOUSSA		IVORY COAST	tjap	U	T	1			1	1
444	YANGKUM RED		BHUTAN	tjap	NA	T	1			1	3
445	YUNLU 7		CHINA	tjap	U	I	1	1	2	1	3
749	CHUAN 3		TAIWAN	tjap	U	NA	1				
750	CHUAN 4		TAIWAN	tjap	U	NA					
18	KHAO DAWK MALI 105		THAILAND	indica	RL	T	1				
	TOTAL	17					190	43		176	

Abstract

Rice, the tropics' foremost food crop, is particularly vulnerable because of its high water demand and poor drought resistance. Early vigour (ie.shoot biomass accumulation) is essential for rapid crop establishment, resource acquisition and can thus contribute to drought avoidance. To take advantage of the genetic diversity for rice improvement, this work aims at characterizing the diversity of morphogenetic, metabolic and hydraulic component traits constituting early vigor and its plasticity under drought. A rice diversity panel of 200 tropical japonica accessions was phenotyped in two greenhouse experiments. Plant early vigor, was detailed into (i) morphogenetic (tillering, leaf size and appearance rate), (ii) metabolic (starch, sucrose, hexose) in source (fully expanded) and sink (expanding, hidden) leaves and (iii) plant water use, related traits. The experimental setup was designed to prevent the expression of genotypic rooting differences.

This study demonstrated that sink dynamics: Developmental Rate (DR, inverse of phyllochron, in °C.d^{-1}); tillering capacity and potential leaf size which together constitute incremental demand for assimilates are mayor drivers of early vigor. A tradeoff between DR and leaf size was explained by differences in carbon concentrations in source and sink leaves, in particular high DR genotypes stored few starch in source leaves compared to large leaf genotypes under well watered conditions. Low drought tolerance was related to a reduction in sink activity under drought. Genotypic differences in sink activity under drought were related to constitutive morphological traits and NSC pool sizes, plant leaf transpiration rates and its control by soil water deficit and to water use efficiency under drought (WUE). Thus, demonstrating that rice has a great genetic diversity in terms of drought tolerance. The study of phenotypic associations provided a typology of the germplasm studied and show that under drought both high DR and large leaves vigorous genotypes had the strongest growth reduction. Indeed, DR was associated to high stomatal sensibility to drought and low WUE, while large leaves genotypes showed high leaf senescence rates.

Finally, the phenotypic diversity observed within the studied japonica panel is promising for genetic studies in order to improve rice drought resistance. The genetic limitations of the negative, phenotypic linkages observed between early vigor and drought tolerance, and thus the easiness to co-select for both traits will have to be explored. For this purpose we conclude on the relevance of dissecting early vigor into component constitutive (observed under well watered) and drought response traits for both metabolic and morphogenetic processes. We pointed out the interest of studying the natural diversity to identify trait combinations leading to different growth strategies. The key role to be played by novel (metabolic, plant) platforms in the future, toward more integrative screening approaches is discussed. Traits explored in this Ph'D work will be included in a genetic association study using Single Nucleotide Polymorphism markers.

Resumé

Le riz, composant de base de l'alimentation humaine dans les tropiques, est une céréale dont les rendements sont limités par de fortes demandes en eau et une faible résistance au stress hydrique. La vigueur initiale (accumulation de biomasse aérienne) est déterminante pour un rapide

établissement de la culture et l'accès aux ressources, contribuant ainsi à un évitement du stress hydrique. Pour profiter de la diversité génétique dans l'amélioration variétale chez le riz, cette thèse vise à caractériser la diversité phénotypique des traits constituant la vigueur initiale et sa plasticité sous stress hydrique, sur la base de caractères morphogénétiques, métaboliques et hydrauliques. Un panel de 200 génotypes de japonica tropicaux a été phénotypé dans deux expériences en milieu contrôlé. La vigueur de la plante a été analysée avec des traits morphogénétiques (tallage, taille et taux d'apparition des feuilles), métaboliques (amidon, saccharose, hexoses) dans les organes sources (feuilles développées) et puits (feuilles en expansion), ainsi que des caractères liés à l'utilisation de l'eau. Le dispositif expérimental à été conçu pour éviter l'expression de différences génotypiques au niveau de l'enracinement et étudier des caractères de tolérance à la sécheresse.

Cet étude à démontrée que la vigueur initiale dépend fortement de caractères relatifs aux forces de puits et à la demande en assimilats carbonés, tels que le taux de développement (DR, inverse du phyllochron, $°Cd^{-1}$), le tallage et la taille potentielle des feuilles. Une relation négative entre DR et taille des feuilles a été démontré et reliée à des différences d'utilisation des sucres par la plante au niveau des organes source et puits. En particulier des plantes à fort DR ont montré la tendance à stocker très peu d'amidon dans les feuilles source, inversement aux génotypes à grande feuilles. Sous stress hydrique des faibles tolérances à la sécheresse ont été liés à des réductions des activités des organes puits. Les différences génotypiques pour l'activité des puits sous stress ont été liés à des différences pour des caractères constitutifs de type morphogénétique et métabolique, aux taux de transpiration foliaire et à son contrôle stomatique sous stress ainsi qu' à l'efficience d'utilisation de l'eau sous stress. Ceci a démontré que le riz possède une diversité génétique en termes de tolérance au stress hydrique. L'étude de combinaisons entre caractères phénotypiques a permis d'élaborer une typologie du panel étudié et a montré que sous stress hydrique les génotypes vigoureux avaient de fortes réductions de croissance. En effet de forts DR étaient aussi associés à une forte sensibilité du taux de transpiration foliaire (fermeture stomatique) et à une faible efficience d'utilisation de l'eau sous stress. Les génotypes à grandes feuilles ont montré un fort taux de sénescence foliaire.

La diversité phénotypique observée dans le panel des riz japonica est prometteuse pour des analyses génétiques permettant l'amélioration de la tolérance au stress hydrique du riz ; cependant, les éventuelles limitations génétiques liées aux relations négatives observées entre vigueur initiale et tolérance au stress hydrique et donc, la facilité d'une co-sélection pour ces deux caractères complexes, devront être explorées. Pour cela, nous concluons sur la pertinence de (i) les disséquer en caractères de nature morphogénétique, métabolique et hydraulique et (ii) d'exploiter des panels de diversité pour mieux appréhender les liaisons existantes entre caractères élémentaires. Le rôle grandissant des plateformes (métaboliques, 'phénomiques'), pour un phénotypage plus intégratif du comportement de la plante entière, est discuté. Les caractères étudiés dans ce travail seront utilisés pour des études de génétique d'association à haut débit sur la base d'une puce de 1 million de marqueurs SNP (Single Nucleotide Polymorphism).